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BULLETIN OF

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ON THE MECHANISM OF THE EFFECT OF ACETYLCHOLINE ON THE PULMONARY CIRCULATION

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)
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Certain contemporary researchers consider acetylcholine to be one of the substances most actively participating in regulation of the vascular tonus in the pulmonary circulation. However, the known difficulties of studying pulmonary circulation and certain features of the experimental methods employed have given rise to contradictory opinions regarding the action mechanism of acetylcholine in this vascular region.

Acetylcholine's effect on the pulmonary vessels has been studied on isolated lung vessels [1, 17], isolated lungs under conditions of perfusion [3, 7, 11] and animals with an exposed thoracic cage [5, 9]. A review of the literature up to 1933 can be found in Daley's work [6].

In the investigations using perfusion of isolated lungs, acetylcholine was found to have vasocontrictor effect in large doses and a vasodilator effect in small doses. Rose reached the same conclusion in a recently published work experimenting on dogs in which the left ventricle was replaced by a perfusion pump [16].

In investigations they carried out on people and animals without exposing the thoracic cage, Cournand and co-workers [4, 13, 14] and Wood and co-workers [18] concluded that acetylcholine exerts an indirect vasodilator effect on the pulmonary vessels. Other researchers [5, 10, 12], however, believe the pressure changes in the pulmonary circulation to be due to redistribution of the blood.

Our preliminary observations [2] demonstrated that acetylcholine is not the immediate cause of changes in the tonus of the pulmonary vessels. The fall of pressure in the pulmonary artery occurs as a result of change in the systemic circulation.

The purpose of this work was to further investigate acetylcholine's effect on the pulmonary circulation.

EXPERIMENTAL METHOD

The experiments were performed on 21 dogs [106 observations with the animals under morphine (0.0025 g/kg)-chloralose (0.05-0.07 g/kg) anesthesia]. The hemodynamic indices were determined with a multichannel water-mercury manometer and synchronously recorded with a kymograph. The pressure in the pulmonary artery and the pulmonary "capillary" pressure (Wedge pressure) were recorded through catheters introduced through the external jugular vein. In some experiments, catheters with a double or triple lumen were used for simultaneous registration of the arterial and "capillary" pressures in the pulmonary circulation. The central venous pressure (pressure in the venae cavae) was recorded through catheters introduced through one of the femoral veins. The hepatic veins were catheterized in three experiments.

Besides the hemodynamic indices, we also recorded the respiratory variations of the pressure in the trachea and, in special experiments, the intrapleural pressure and the oxygen saturation of the arterial blood in the carotid artery, using a circulating device for the latter. Catheterization of the cardiac cavities and trunk vessels was also used for the administration of acetylcholine. The catheters were introduced through the carotid artery into the aorta and the left ventricle of the heart. In every case, the position of the catheters was determined by the level of the pulse and respiratory pressure waves and by x-ray, and then verified on sections at the end of each experiment.

In addition to the experiments on intact animals, we also performed experiments on dogs with an exposed thoracic cage, using autoperfusion of a vascularly isolated lung lobe with venous blood and recording the pressure in the left auricle.

EXPERIMENTAL RESULTS

Acetylcholine was administered in doses of 0.5-50 γ/kg animal weight. After the administration of acetylcholine in small doses (0.5-5 γ/kg), the pressure in the systemic circulation and the cardiac rhythm did not change in many experiments, but the pressure in the pulmonary artery always rose.

When acetylcholine was administered into veins of the systemic circulation, the pressure in the pulmonary artery rose 12-14 sec later, but the pressure rise occurred after 7.8 (4-11) sec when the substance was introduced into the pulmonary artery and after 4 (2-7) sec when it was introduced into the left ventricle and aorta.

The degree and duration of the pressure rise depended on the dose of the substance administered. There was a greater and more prolonged rise of pressure in the pulmonary artery after the administration of acetylcholine into the left ventricle and arch of the aorta than after the administration of the same dose into a vein of the systemic circulation or directly into the pulmonary artery (Figs. 1, 2).

The average pressure rise in the pulmonary artery was 28.2 (16-48) mm of water following the introduction of acetylcholine into the femoral vein or pulmonary artery, but 40 (25-66) mm of water following its introduction into the aorta. The pressure in the pulmonary circulation retumed to the original level after 1-3 min in the majority of observations.

Regardless of where acetylcholine was introduced, a decrease of pressure in the systemic circulation was observed either before or, as in several cases, almost simultaneously with the pressure rise in the pulmonary circulation. The cardiac rhythm was usually accelerated. When large doses were administered, especially into the left ventricle and arch of the aorta, acute retardation of the rhythm was observed at the beginning of the reaction. In these cases, there was an acute fall of pressure in the systemic circulation, while there was only a brief decrease of the pressure in the pulmonary artery, followed by its increase.

The pulmonary "capillary" pressure was recorded in five experiments (24 observations). The administration of acetylcholine in small doses did not cause much change in the capillary pressure. The considerable acceleration of cardiac activity following the administration of acetylcholine in large doses was attended by a fall of capillary pressure (Fig. 3). The pressure gradient between the pulmonary artery and the lung capillaries rose in every case.

The two experiments (six observations) performed on dogs with an exposed thorax in order to record the pressure in the left auricle showed little change in the latter. There was usually a fall of pressure in the venae cavae and right auricle, sometimes preceded by a slight rise. An increase was observed in the pressure recorded after a catheter was wedged into a small hepatic vein. The administration of small doses caused no change in the latter pressure.

Large doses of acetylcholine caused a slight increase in the inflow pressure and a decrease in the outflow in a lung lobe being perfused with venous blood, the nerve connections being intact.

The respiratory changes were variable. In most cases, we observed respiratory arrest followed by stronger, deeper respiratory movements. In a few experiments, there was no apnea preceding this intensification of respiration. The administration of acetylcholine in small doses caused little change in the respiration.

The administration of acetylcholine in a dose of 10 γ/kg (21 observations) caused little change in the tonus of the bronchial musculature as observed during the synchronous recording of the respiratory fluctuations

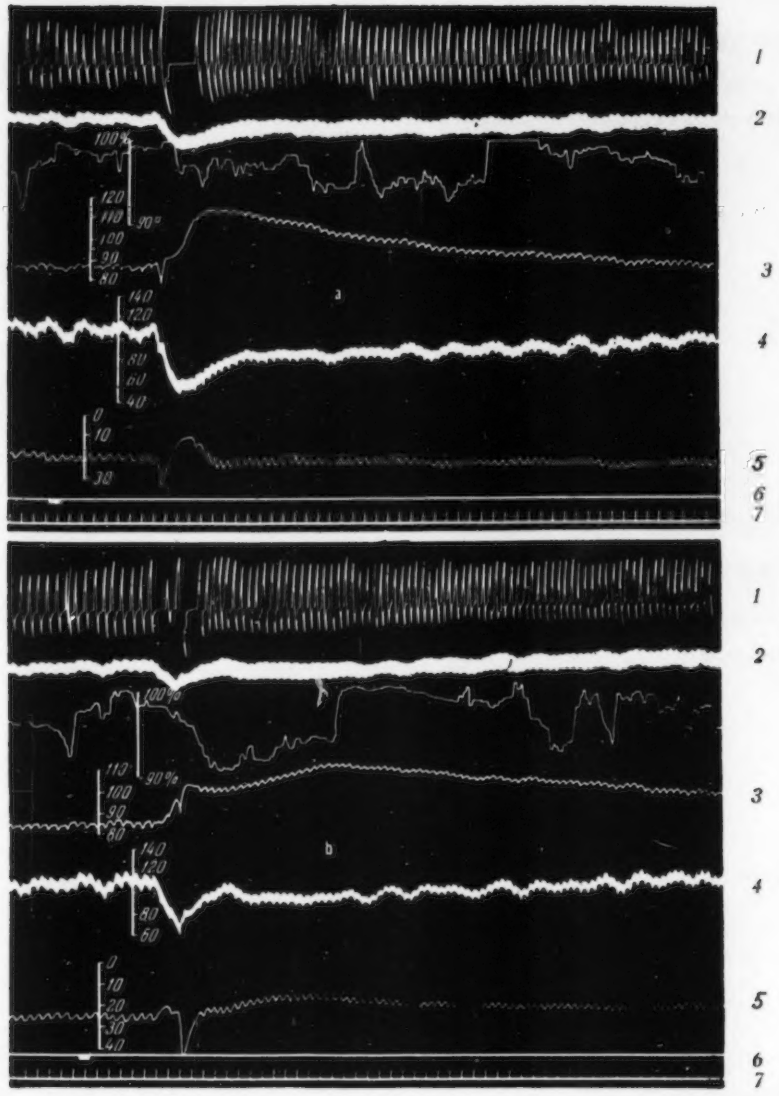

Fig. 1. Reaction to introduction of acetylcholine into pulmonary artery (a) and into thoracic aorta (b). Male dog weighing 24 kg, morphine-chloralose anesthesia. Acetylcholine dose, 10 γ/kg. Curves show: 1) respiration; 2) pressure in femoral artery (membrane manometer); 3) average pressure in pulmonary artery (in mm of water); 4) pressure in femoral artery (in mm of mercury); 5) average pressure in inferior vena cava (in mm of water); 6) indication of stimulation; 7) time in 5-second marks.

and the intrapleural and intratracheal pressures. Acetylcholine's bronchospastic effect was much stronger on a background of neostigmine administration (Fig. 4).

We could not, however, observe any direct relationship between the degree of pressure rise in the pulmonary artery and the intensity of the bronchospasm. The rise of pressure in the pulmonary circulation also occurred in the absence of bronchospasm.

Prompted by the communications of Cournand and co-workers [4, 13, 14] concerning the stronger vasodilator effect exerted by acetylcholine under conditions of hypertonia of the pulmonary circulation, we set up experiments analogous to theirs.

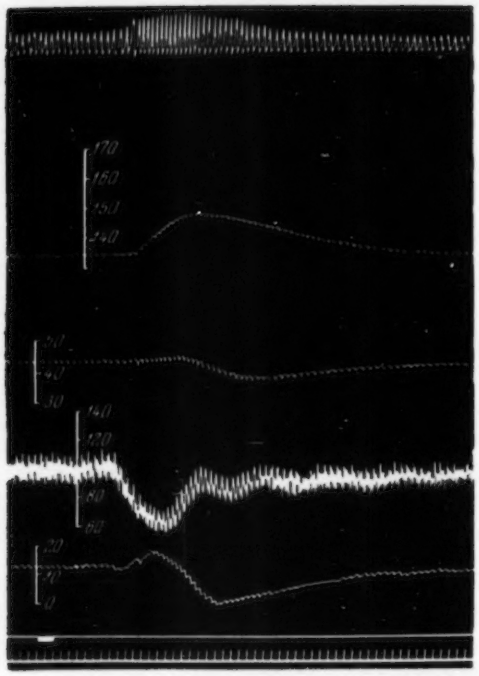

Fig. 2. Reaction to introduction of acetylcholine into pulmonary artery. Male dog weighing 23 kg, Amytal anesthesia. Acethylcholine dose, 10 γ/kg. Curves show (from top to bottom): respiration; average pressure in pulmonary artery (in mm of water); average lung capillary pressure (in mm of water); pressure in femoral artery (in mm of mercury); average pressure at mouth of venae cavae (in mm of water); indication of stimulation; time in 3-second marks.

A rise of pressure in the pulmonary circulation of four dogs (19 observations) was induced by giving the dogs gaseous mixtures with a low oxygen content (6-8%) to inhale. Under these conditions, the pressure in the pulmonary artery rose 20-50%, and other compensatory reactions typical of hypoxia developed: the pressure in the systemic circulation rose; intensified cardiac activity was observed. The compensatory faculties of the organism diminished toward the end of the first or the beginning of the second hour of hypoxia: the blood pressure gradually decreased; stronger tachycardia and decreased pulse pressure were observed; waves of the third order appeared; and the palpebral reflexes diminished and periodically disappeared.

The reactions to acetylcholine changed as hypoxia increased.

The typical reaction to acetylcholine, i.e., a rise of pressure in the pulmonary artery, was retained during the period of stable compensation with increased blood pressure and moderate tachycardia. In three observations, the pressor reaction was even enhanced. As the hypoxia condition developed and the blood pressure in the systemic circulation decreased, the pressor effect in the pulmonary circulation induced by acetylcholine became gradually weaker and then distorted. Under these conditions, the rhythm of the heart activity became retarded during the reaction period. The direction of the reactions of the systemic circulation remained the same. The respiration became faster and deeper with no preliminary apnea.

We next analyzed the reaction to acetylcholine after preliminary administration of hexamethonium (4-8 mg/kg) or atropine (0.5 mg/kg) and after transection of the vagosympathetic nerves to the neck.

In both the pulmonary circulation and the systemic, the reaction to acetylcholine was less pronounced after the preliminary administration of hexamethonium. Vagotomy did not diminish the reaction to acetylcholine.

In atropinized animals, the intravenous administration of average doses of acetylcholine had either a sharply reduced or an almost nonexistent effect. However, acetylcholine introduced by catheter into the left ventricle and aorta caused a well-expressed reaction.

Therefore, it has been demonstrated that the passage of acetylcholine through the pulmonary vascular bed is not attended by recordable pressure changes in the pulmonary circulation.

A pressor reaction was observed 4-11 sec (an average of 7-8 sec) after the injection of the drug directly into the pulmonary artery. The introduction of acetylcholine into the left ventricle and into the aorta also induced a rise of pressure in the pulmonary circulation which occurred earlier (after 4 sec) than that following the introduction of the drug into the femoral vein or directly into the pulmonary artery. The pressor reaction induced by the introduction of acetylcholine into the left ventricle and aorta was more pronounced than that caused by the administration of the drug into the venous section of the systemic circulation. The initial fall of pressure in the pulmonary artery preceding its rise is due solely to inhibition of the cardiac activity and can be easily reproduced by administering acetylcholine into the pulmonary artery and left ventricle of the heart.

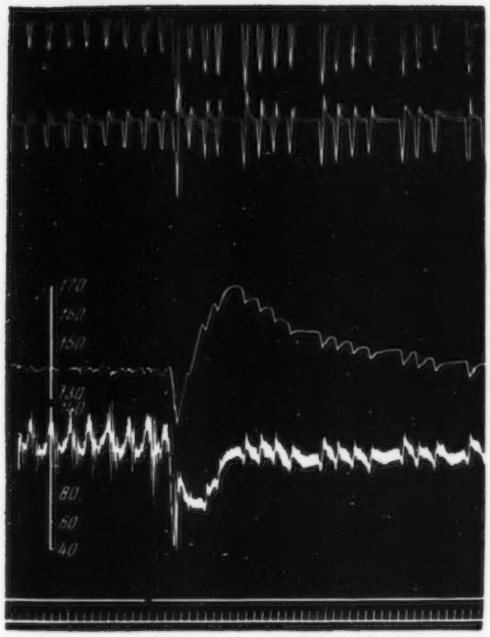

Fig. 3. Reaction to introduction of acetylcholine into femoral vein. Male dog, morphine-chloralose anesthesia. Acetylcholine dose, 10γ/kg. Curves show (from top to bottom): intrapleural pressure; respiration; average pressure in pulmonary artery (in mm of water); pressure in femoral artery (in mm of mercury); indication of stimulation; time in 3-second marks.

It should be definitely established that acetylcholine does not directly cause changes in the hemodynamics of the pulmonary circulation and does not affect the tonus of the pulmonary vessles. The time required for the development of the pulmonary circulation's reaction and the fact that the character of the reaction is the same whether acetylcholine is administered into the venous or arterial section of the vascular bed give reason to believe that the hemodynamic effects in the pulmonary circulation merely reflect the hemodynamic changes in the systemic circulation. The changes which occur in the reaction of the systemic circulation under conditions of hypoxia or experimental hypertonia of the pulmonary circulation are reflected to the same extent by the reaction of the pulmonary circulation.

The influence of the so-called reverse transfer of pressure from the left auricle in the mechanism of acetylcholine's pressor effect in the pulmonary circulation can also be completely negated. The pressure changes in the left auricle are much less pronounced than those in the system of the pulmonary artery and sometimes in the opposite direction.

Acetylcholine is known to raise the tonus of the bronchial musculature considerably. In Rodbart's opinion [15], spasm of the bronchioli causes the intraalveolar pressure and capillary resistance to increase.

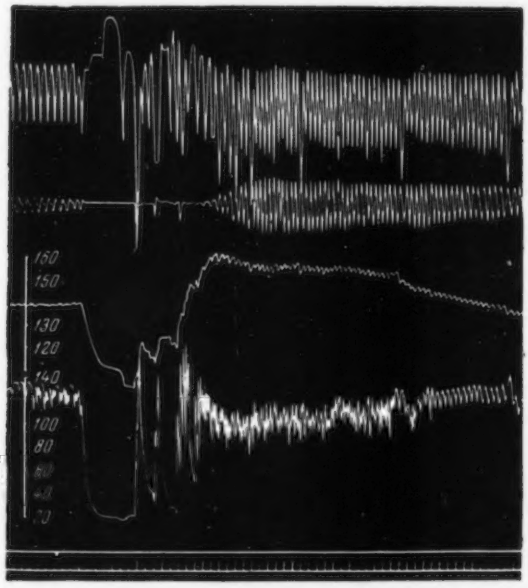

Fig. 4. Reaction to introduction of acetylcholine into femoral vein on a background of neostigmine action. Symbols the same as in Fig. 3.

However, a rise of pressure in the pulmonary artery can also occur without any changes in the bronchomotor tonus. Rose also [16] was unable to detect any direct relationship between changes in the tonus of the bronchial musculature and hemodynamic changes in the pulmonary circulation.

The above analysis gives reason to believe that the pressure rise effected by acetylcholine in the pulmonary artery is basically a secondary result of hemodynamic changes in the systemic circulation. After the administration of acetylcholine, a complex of reflex compensatory reactions develops immediately after dilatation of the systemic vessels to counteract the fall of arterial pressure and acetylcholine's effect on the vascular interoceptors. Cardiac activity is accelerated, the pulse pressure rises and the outflow from the portal vein system increases, probably because of the contraction of the muscles of the gastrointestinal tract. Under these conditions, the pressure gradient between the pulmonary arteries and the capillaries also increases. In all probability, this is due to reflex changes in the tonus of the pulmonary vessels as well as to the increase in the inflow.

SUMMARY

It was shown in experiments on dogs that during the circulation through the pulmonary vascular system of acetylcholine added to the blood no recordable pressure changes were noted in the pulmonary circulation. Pressor reaction began in 4-11 seconds after the administration into the pulmonary artery. Injection of acetylcholine into the left ventricle and aorta also leads to the pressor reaction in the pulmonary circulation; this occurs earlier than when the substance is injected into the femoral vein or directly into the pulmonary artery (in 4 seconds). Besides, pressor reaction is more marked in administration of acetylcholine into the left ventricle and aorta than in injection into the venous part of the systemic circulation. Pressure reduction in the pulmonary artery, preceding its rise, is directly connected with the inhibition of the cardiac activity and is also well reproduced with acetylcholine administered into the pulmonary artery and the left ventricle of the heart.

Thus, acetylcholine does not provoke direct changes in the hemodynamics of the pulmonary circulation, and, evidently, does not influence the tone of the pulmonary vessels.

Period of reaction development in the pulmonary circulation and the same type of reaction occurring in acetylcholine administration into the venous and the arterial portion of the circulation lead to the conclusion that the hemodynamic effects the pulmonary circulation reflect the hemodynamic shifts in the systemic circulation.

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THE CONDITIONED REFLEX ACTIVITY OF DOGS BEFORE AND AFTER BILATERAL EXTIRPATION OF THE PARIETAL CORTEX

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(Presented by Academician V. N. Chernigovskii)
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There are few reports in the literature of research into the conditioned reflex activity of dogs after extirpation of the parietal region on one side. From the little information available [5, 6, 8], according to which the parietal region of the dog takes part in the analysis and integration of simple sound and light stimuli and mechanical stimuli of the skin, it is difficult to judge the character of the localization of these functions, for in all these cases the parietal region was removed together with other regions of the cortex (occipital, temporal, postcoronary). Removal of the parietal lobes [7] led to the temporary (5-7 days) disappearance of the conditioned motor reflexes to a sound stimulus. A study which we made of the efferent pathways of the parietal region of the dog [3] showed that from this point of view this region is complex – it has projection connections with those subcortical structures with which other regions of the cortex are connected, especially the precoronary, occipital and postcoronary regions.

As a consequence of the foregoing considerations, we studied certain functional properties of the parietal region of the dog.

EXPERIMENTAL METHOD

We studied the conditioned reflex activity in the experimental animals before and after the bilateral extirpation of this particular region (Area 5 and Area 7).*

The investigation was carried out on two dogs (Kashtanka and Belyak). In the course of the work we studied the motor reactions of the dogs when allowed to move about freely in a chamber [1]. We used simple sound and visual stimuli and mechanical stimuli of the skin as conditioned stimuli. The parietal cortex was removed in two stages – the parietal region of one hemisphere was first removed, followed 2-3 weeks later by that from the other hemisphere. The dog was sacrificed four months after the second operation.

EXPERIMENTAL RESULTS

The dog Kashtanka. The conditioned motor reaction to a positive sound stimulus – bell 1 – appeared after eight reinforcements. Differentiation to bell 2 was consolidated after the 52nd application. The conditioned motor reaction to a positive light stimulus – white light 1 from a 100 w lamp – appeared and became constant after seven reinforcements. Differentiation of the light stimuli – light 9 – was consolidated after six applications

* We have studied the cytoarchitectonics of these areas and compiled a cytoarchitectonic map of this region [2].

Fig. 1. Character of the conditioned reflex activity of the dog Kashtanka before the first operation. Significance of the curves (from above down): marker of the dog's visit to the feeding bowl; marker of the dog's visit to the sleeping corner; marker of application and duration of action of the conditioned stimulus; marker of presentation of the feeding bowl for reinforcement of the conditioned stimulus by an unconditioned; automatic time marker (1 second). b_1) Bell 1; b_2) bell 2; l_1) light 1; l_9) light 9; t_1) touch 1 (right thigh); t_2)touch 2 (right shoulder); ws) white square (+); wt) white triangle (−).

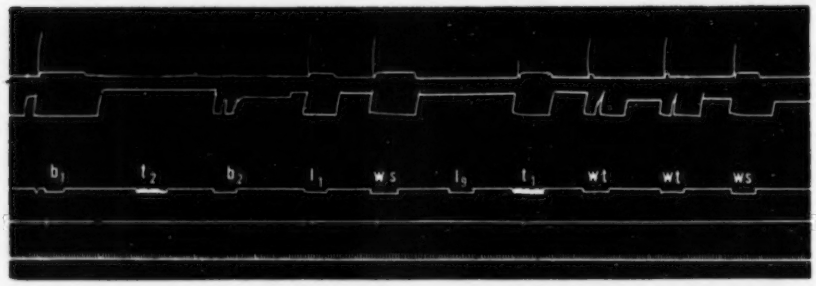

Fig. 2. Character of the conditioned reflex activity of the dog Kashtanka before the second operation. Legend as in Fig. 1.

of the stimulus (the brightness of the light stimulus was determined by the degree of blackening of an x-ray film placed in the path of the beam of light in the form of a filter). The positive conditioned reflex to mechanical stimulation of the skin − touch stimulus 1 in the region of the right thigh − appeared at the 26th combination and was consolidated after 51 applications. Differentiation in the form of touch stimulus 2 in the region of the right shoulder appeared after four applications of the stimulus. In addition an attempt was made to produce reflexes to objective stimuli: positive − a white square, and negative − a white triangle. The kymograph tracing shown above gives an idea of Kashtanka's conditioned reflexes before the first operation (Fig. 1).

The parietal cortex from Kashtanka's left cerebral hemisphere was then ablated. After the operation the dog reacted in a lively manner to surrounding stimuli, pricking the skin of the limbs was accompanied by their withdrawal, and no abnormality of the gait was observed. On the third day after operation Kashtanka took part in the experiment. It was found that the animal's conditioned reflex activity was essentially unchanged: the dog reacted to all the positive stimuli that were applied by a movement towards the feeding bowl; when the differential stimuli were applied the dog remained in its initial place. After the first operation the production of differentiation to the white triangle was continued. The animal no longer walked to the feeding bowl after the third application of the triangle. Differentiation to the triangle, however, was unstable, sometimes disappearing and then subsequently reappearing.

In Fig. 2 we show a tracing of the conditioned reflex activity of the same dog before the second operation. On the day after the second operation (extirpation of the parietal cortex of the right hemisphere) the dog was lethargic, moved about very slowly, placed its hind limbs wide apart, and performed a series of sliding movements with them on the floor. There was a slight predominance of the tone of the extensor muscles of the limbs. Vision was disturbed, and the dog often collided with objects on its left side. No disturbances of pain sensation

were observed. On the third day after operation Kashtanka took part in the experiment. The conditioned reflexes and differentiation to all the stimuli applied were preserved. Motor disturbances, however, were present – the dog slowly raised itself up to the feeding bowl and its hind limbs slipped about the floor; disturbances of vision were also observed. From the sixth day after operation the above-mentioned motor disturbances and the disturbances of vision began to disappear gradually. In later experiments no changes in the conditioned reflex activity were likewise found.

In order to test the strength of the process of inhibition in the dog after extirpation of the parietal cortex, the application of the differential sound stimuli (bell 2) was specially prolonged to two minutes (instead of the usual 20 seconds). This procedure caused no appreciable disturbance of the conditioned reflex activity. The positive sound stimulus (bell 1) was then extinguished. The conditioned motor reaction to this stimulus did not appear after 25 failures to reinforce the bell 1. During this period no significant disturbances of the conditioned reflex activity were observed in the dog. Kashtanka was sacrificed four months after the second operation. During this period no changes were found either in the conditioned reflexes which we studied or in the dog's behavior.

In the second dog (Belyak), as in the first (Kashtanka), conditioned reflexes were produced to sound (bell 1 and bell 2) and light stimuli (light 1 and light 9) and to mechanical stimulation of the skin (touch stimuli 1 and 2). The conditioned motor reflex to bell 1 appeared after 13 combinations with food reinforcement; differentiation to bell 2 became stable after 45 applications. The motor conditioned reaction to light 1 appeared after six combinations, and the negative reaction to light 9 after two applications. The conditioned reflex to touch stimulus 1 appeared after three combinations, and differentiation to touch stimulus 2 at the third application.

The parietal cortex on both inner and outer surfaces of the right hemisphere of the dog Belyak was then ablated, and the procedure was repeated on the left side 20 days later.

Neither the first nor the second operation caused changes in the previously produced conditioned reflexes (Fig. 3). As in Kashtanka, however, motor disturbances were observed in Belyak (the dog moved about slowly, widely separated its hind limbs and performed sliding movements) together with disturbances of vision (the dog collided with objects situated on the side opposite the site of operation). Cutaneous sensation could not be tested because of the dog's marked aggressiveness. The above-mentioned disturbances were very clearly noticeable for 5-6 days after the operation after which they gradually began to disappear.

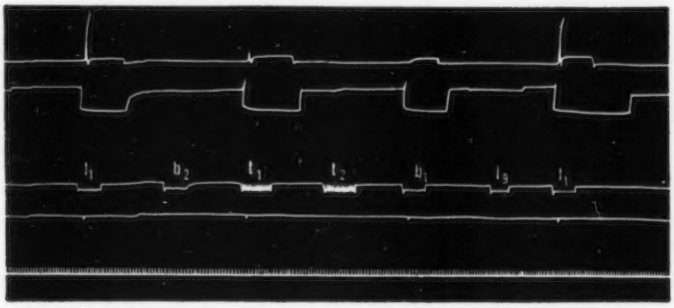

Fig. 3. Character of the conditioned reflex activity of the dog Belyak on the fourth day after the second operation. Legend as in Fig. 1.

In the dog Belyak, as in Kashtanka, the action of the differential sound stimulus (bell 2) was deliberately prolonged, and this was followed by extinction of the positive sound stimulus (bell 1). These procedures caused no change whatever in the conditioned reflex activity of the dog Belyak.

When our findings are compared with the results of investigations by other workers, it must noted that similar phenomena were described by Bekhterev [4] and Peele [9] in association with lesions of the parietal region in the dog and the monkey. The disturbances of pain and tactile sensation observed by Bekhterev may be attributed to the fact that he removed not only the parietal cortex but also the postcoronary region. So far as the other disturbances (movement, vision) are concerned, as our investigations showed these are evidently associated with the destruction of the parietal region (although the effect of postoperative trauma cannot be excluded).

After extirpation of the parietal region in the dog we thus were unable to detect changes affecting the conditioned reflexes, investigated during free movement of the animal, to simple sound and light stimuli and to mechanical stimulation of the skin. At the same time the motor and visual disturbances which we observed after bilateral ablation of the parietal region may indicate that the parietal cortex participates in the functions of both the motor and the optic analyzers. This view is in agreement with our previous findings [3] that various areas of the parietal region are interconnected, namely: Area 7 is connected by efferent fibers with the posterior part of the lateral nucleus of the thalamus, with the superior colliculus and with the nuclei of the pons; Area 5 sends efferent fibers into the medial lamina of the globus pallidus, into the middle part of the lateral nucleus of the thalamus, into the lateral part of the substantia nigra, into the nuclei of the pons and into the spinal cord.

On the basis of our findings relating to the functional characteristics and the connections of the parietal region in the dog it may be postulated that scattered elements of the optic and motor analyzers are situated in the parietal cortex.

SUMMARY

Conditioned reflexes were examined in two dogs allowed to move freely in response to sound, light, and skin-mechanical stimuli. Both the unilateral and bilateral removal of parietal area provoked no significant disturbances in the conditioned reflex activity of dogs. However, in bilateral extirpation of this area motor and visual disturbances were observed. These data as well as evidence obtained earlier and relating to the connections in this area lead to a suggestion that the parietal area participates in the function of the motor, as well as of the visual, analyzer.

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THE GAS EXCHANGE IN EXPERIMENTAL HYPOTHALAMIC ADIPOSITY

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A bilateral, symmetrical lesion of the hypothalamus in the region of the ventromedial nuclei or of regions lying immediately lateral thereto causes the development of adiposity in rats [3, 5, 6, 8, 10]. In the pathogenesis of this type of experimental adiposity considerable importance is attached to the onset of hyperphagia. This may be regarded as the result of the fact that the destruction of these nuclei releases the function of the ventrolaterally situated nerve formations, composing an important section of the food center, from the regulating (inhibitory) control [3, 6, 7]. A decrease in the mobility of the animals is of definite importance.

An increase in the respiratory coefficient above unity, observed in rats with hypothalamic adiposity after taking food or administration of glucose, demonstrated the intensification of the conversion of carbohydrates into lipids. Similar values of the respiratory coefficient were observed in rats without lesions of the hypothalamus, if by means of suitable training the animal was made to eat the whole of its day's ration in a short period. From this fact it has been suggested [6, 9, 10] that the increase in the respiratory quotient in "hypothalamic" rats is not the result of a primary disturbance of metabolism, but a secondary phenomenon due to hyperphagia.

We can hardly accept the view that hyperphagia is the principal factor concerned in the pathogenesis of hypothalamic adiposity. The adiposity persists in animals for a long time after the infliction of injury on the hypothalamic region, when the hyperphagia, which is well marked during the first few days and weeks after injury, is considerably weakened. The disturbance of the activity of the hypothalamic nerve formations is bound to influence the trophic processes associated with lipid metabolism. Diencephalic adiposity in man, of which experimental hypothalamic adiposity is a reproduction, is accompanied by no means invariably by hyperphagia.

It was therefore considered necessary to study the state of the basal metabolism and the value of the respiratory quotient in experimental hypothalamic adiposity in connection with the quantity of food eaten, and also the change in these indices in the obese animals during fasting and in response to the injection of glucose and amino acids.

EXPERIMENTAL METHOD

Experimental adiposity was induced in rats by bilateral electrolytic destruction of the region of the median eminence of the hypothalamus at the site of the ventro- and dorsomedial nuclei. The destruction was carried out by means of a stereotaxic apparatus devised by Belenev and Kabak [1]. Rats weighing 170-230 g were anesthetized with nembutal, and through an opening in the parietal bone an electrode (the anode) was introduced, the angled tip of which was situated approximately 1.8-2.0 mm posterior to the bregma, 0.5-0.6 mm lateral to the midline and 0.4 mm from the base of the skull. The cathode was connected by a wet contact to the animal's tail. A current of 2 ma was passed for 5-30 seconds (for a detailed description of the method see [1]). After

TABLE 1

Gas Exchange in Rats with Hypothalamic Adiposity

Rat No.	Date of injury	Initial wt. (in g)	Date of investigation (1959)	Wt. at the time of investigation (in g)	O_2 consumption (in 1 hr)		Excretion of CO_2 (in 1 hr)		Respiratory quotient
					absolute (liters)	per cm^2 of body surface (in ml)	absolute (liters)	per cm^2 of body surface (in ml)	
Control group – 10 rats (mean figures)		—	—	208	0.37	1.16	0.35	1.10	0.94
79 ♀	Jan. 20, 1959	182	Mar. 28, April 7	312—322	0.45	1.05	0.47	1.10	1.04
6 ♀	Dec. 17, 1958	280	April 1, May 13	340—386	0.62	1.28	0.62	1.28	1.0
15 ♂	Feb. 20, 1959	205	July 24, Aug. 31 Nov. 21	482—497 546	0.53 0.43	0.92 0.70	0.60 0.63	1.04 1.03	1.13 1.46
16 ♂	Feb. 20, 1959	211	July 23 Aug. 1 Aug. 15 Aug. 18	531 528 540 533	0.37 0.45 0.54 0.56	0.61 0.76 0.89 0.93	0.48 0.45 0.70 0.70	0.80 0.76 1.16 1.17	1.29 1.0 1.3 1.25
46 ♂	March 20, 1959	220	July 22, Aug. 17 Aug. 18, Aug. 24 Nov. 16, Nov. 20	686—700 703—680 646—654	0.55 0.62 0.59	0.77 0.87 0.86	0.60 0.58 0.73	0.84 0.81 1.06	1.09 0.93 1.24
24 ♀	June 27, 1958	170	Mar. 14, Mar. 23 Mar. 23, April 8	436—406 406—461	0.48 0.55	0.96 1.01	0.52 0.69	1.03 1.27	1.08 1.25
4 ♀	June 30, 1958	165	Mar. 13 Apr. 19	570 606	0.57 0.54	0.91 0.83	0.68 0.72	1.08 1.10	1.19 1.33
1 ♀	May 28, 1958	177	Feb. 28, Mar. 20	725—784	0.57 (0.51—0.63)	0.73	0.73 (0.68—0.78)	0.94	1.28 (1.25—1.36)

the operation the animals were kept for 1-2 days in separate cages; they were given food and water next morning. The rats developed a considerable hyperphagia very quickly after the operation, although its appearance during the first few days after the operation did not, in our observation, demonstrate the inevitable development of adiposity.

Investigation of the gas exchange for a period of one hour by the closed chamber method in an apparatus for small animals (Shaternikov's type, as modified by Isichenko [2]) was carried out 3-11 months after operation. The weight of the rats at this period was considerably above normal, and in one female (No. 1) it reached 784 g. At the end of the investigation the animals were sacrificed and serial histological sections of the hypothalamus were made (fixation with formalin, stained with thionin). We studied every tenth section, cut to a thickness of 10 μ. In all the adipose rats investigated we found bilateral lesions of the hypothalamus with total or partial destruction of the region of the ventro- and dorsomedial nuclei. In determining the site of the lesions we used the pictures from Szentagotai's atlas.

EXPERIMENTAL RESULTS

It will be seen from the figures in Table 1 that the absolute quantities of O_2 absorbed and CO_2 eliminated by the adipose rats were higher, but when calculated per cm^2 body surface (according to the formula: $S = 9.1\ W^{2/3}$, where S is the surface area of the body in cm^2 and W the body weight in g), lower than in the control animals of normal weight. The change in the respiratory quotient is characteristic: in the rats with only slight adiposity (Nos. 79, 6) it was equal or very close to unity; the result was the same in animals with well developed adiposity at those periods when weight was not increased or when it was decreased (rats Nos. 46, 24). At those periods when increase in weight was taking place the respiratory quotient was always greater than unity. In this respect rat No. 1, with well marked and increasing adiposity (see figure) is very demonstrative. At autopsy of this animal the region of damage to the hypothalamus was found to be more extensive than in the other rats (from the middle of the hypothalamus forward, including the anterior part of the ventro- and dorso-premamillary nuclei).

Change in weight in experimental hypothalamic adiposity in rats. On the right – control. On the left – experimental animal (initial weight 177 g, injured on May 29, 1958; weight on February 27, 1959 – 738 g).

It may be concluded from the results obtained that in animals with slight or moderate adiposity there is a preferential utilization of carbohydrates and, at the same time, economy in the utilization of fat. In severe adiposity, in the period of increase in weight, the respiratory quotient greater than unity testifies to the activation of the processes of conversion of carbohydrates into lipids. The same result was obtained during an investigation of the gas exchange in mice with experimental adiposity caused by aurothioglucose [4].

It must be emphasized that in rats in the stage of development of a relatively stable adiposity, with a respiratory quotient greater than unity, no hyperphagia was observed. In rat No. 16, for instance, the body weight remained unchanged for a period of seven days (526-528 g), and the quantity of edible standard diet taken in the 24 hours was on the average 31 g. In the same experimental conditions, in rat No. 15 (weight 490-500 g)

TABLE 2

Change in Gas Exchange in Control Rats and in Rats with Hypothalamic Adiposity during Fasting and Subsequent Feeding

Rat No.	Experimental conditions	Wt. of rat (in g)	Change in weight (in %)	O_2 consumption (in liters per hr)	O_2 elim. (in liters per hr)	Respiratory quotient
	Control					
	Feeding	306	—	0.55	0.50	0.91
	Fasting 24 hr	284	−7.1	0.58	0.49	0.84
	Fasting 48 hr	275	−10.1	0.68	0.55	0.81
	Feeding	290	+5.4	0.57	0.57	1.0
	Experiment					
15 ♂	Feeding	497	—	0.53	0.60	1.13
	Fasting 24 hr	485	−2.4	0.54	0.57	1.05
	Fasting 48 hr	472	−5.03	0.51	0.54	1.06
	Feeding 24 hr	484	+2.5	0.46.	0.61	1.32
	Feeding 48 hr	490	+3.7	0.50	0.55	1.1
1 ♀	Feeding	772	—	0.58	0.80	1.38
	Fasting 24 hr	733	−5.05	0.73	0.92	1.26
	Fasting 48 hr	708	−8.3	0.76	0.93	1.22
	Feeding 24 hr	739	+4.4	0.47	0.80	1.7
16 ♂	Feeding	533	—	0.56	0.70	1.25
	Fasting 24 hr	524	−1.7	0.56	0.64	1.14
	Fasting 48 hr	509	−4.5	0.57	0.62	1.09
	Fasting 3 days	496	−7.1	0.60	0.64	1.06
	Feeding 24 hr	498	−0.4	0.55	0.59	1.07

the daily food intake was 30 g, and in rat No. 4 (weight 658-665 g) the daily food intake was 28.5 g. In the control group of rats (weight 219-225 g) the mean quantity of edible food taken in the 24 hours varied from 28 to 30 g. This suggests that in the stable stage of adiposity hyperphagia is not a factor maintaining the superfluous weight; the reason why it persists is evidently the excessive conversion of carbohydrates into lipids, since the respiratory quotient in these adipose rats was greater than unity, whereas in the control (non-adipose) rats it was less than unity.

The change in body weight of the adipose rats during fasting, as an index of the mobilization of fat, and also the changes in the value of the respiratory quotient in the period of fasting and during the subsequent reversion to normal feeding, are also of interest. In the rats with hypothalamic adiposity, the decrease in weight during fasting for 48 hours was less marked, when expressed in percent, than in the control group (Table 2). This may serve as an indirect index of the inhibition of mobilization of fat in the adipose animals. In the adipose rats with a respiratory quotient greater than unity, the value of the latter diminished during fasting without, however, falling below 1. This fact shows that even in the absence of intake of carbohydrates into the body, the conversion of endogenous carbohydrates into lipids takes place in the adipose animals.

Attention is drawn to the considerable increase in the respiratory quotient when the rats resume normal feeding after preliminary fasting (rats Nos. 15 and 1), if at the same time they increase in weight. When on resuming normal feeding their weight does not increase (rat No. 16), no increase in the respiratory quotient takes place. This variant of the experiments thus also confirms the fact that excessive conversion of carbohydrates into lipids is a factor in the gain in weight during hypothalamic adiposity.

The subcutaneous injection of glucose (0.3 ml of a 40% solution per 100 g body weight) into rats of the control group of normal weight leads in the course of one hour to an increase in the respiratory quotient (Table 3), and in some experiments its value is greater than unity. In adipose rats the injection of the same dose of

TABLE 3

Gas Exchange in Control Rats and in Rats with Hypothalamic Adiposity before and after Subcutaneous Injection of Glucose

Rat No.	Date of infliction of lesion	Date of experiment (1959)	Wt. of rat (in g)	Before injection of glucose			1 hr after injection of glucose				Notes
				O_2 consumption (in liters per hr)	elimination of CO_2 (in liters per hr)	respiratory quotient	dose of glucose (in ml 40% solution per 100 g body weight)	O_2 consumption (in liters per hr)	elimination of CO_2 (in liters per hr)	respiratory quotient	
Control (5 rats, mean figures)											
			200	0.36	0.34	0.94	0.3	0.32	0.34	1.06	
Experiment											
79	Jan. 20, 1959	Mar. 28	312	0.43	0.43	1.0	0.3	0.36	0.43	1.2	
24	June 27, 1958	Mar. 25	440	0.55	0.60	1.09	0.3	0.51	0.60	1.18	
1	May 28, 1958	Feb. 28	784	0.57	0.73	1.28	0.3	0.48	0.78	1.6	
15	Feb. 20, 1959	Aug. 18	492	0.55	0.56	1.02	0.6	0.59	0.61	1.03	Determination after 2 hours
		Aug. 25	510	0.59	0.56	0.95	0.3	0.52	0.55	1.06	
16	Feb. 20, 1959	July 23	531	0.39	0.51	1.3	0.3	0.38	0.51	1.3	
		Aug. 15	540	0.55	0.71	1.3	0.6	0.46	0.64	1.5	
4	June 30, 1958	Mar. 21	595	0.52	0.62	1.2	0.3	0.52	0.63	1.21	Determination after 1 hour
		May 19	606	0.54	0.76	1.4	0.6	0.59	0.75	1.27	
								0.46	0.75	1.63	Determination after 2 hours
46	Mar. 20, 1959	July 22	686	0.48	0.50	1.04	0.3	0.45	0.50	1.1	

TABLE 4

Absorption of Oxygen in Control Rats and in Rats with Hypothalamic Adiposity before and after Injection of 0.3 ml of 5% Cystein Solution per 100 g Body Weight

Rat No.	Date of infliction of lesion	Date of expt. (1959)	Wt. of rat (in g)	Absorption of O_2 (in liters/hr)	
				before injection	after injection
Control group (8 rats, mean figures)			220	0.42	0.50
79	Jan. 20, 1959	Apr. 7	320	0.48	0.57
15	Feb. 20, 1959	July 28	473	0.45	0.45
16	Feb. 20, 1959	Aug. 1	530	0.45	0.45
4	June 30, 1959	April 6	606	0.68	0.70
46	Mar. 20, 1959	July 27	670	0.46	0.43
1	May 28, 1959	April 8	714	0.56	0.56

glucose per 100 g body weight may be accompanied by a fairly well marked increase in the respiratory quotient (see Table 3, rats Nos. 79 and 24 – period of gain in weight, rat No. 1 – well marked adiposity); in other cases – an increase in the respiratory quotient which may be detected during the injection of a large dose of glucose (rat No. 16) or two hours after the injection (rats Nos. 4 and 15). In one rat (No. 46) with well marked adiposity the respiratory quotient was unchanged. These results show that in the stable stage of adiposity, glucose when injected parenterally is either not utilized (the absorption of O_2 and elimination of CO_2 are unchanged) or is partially converted into lipids (the respiratory quotient is greater than unity and the O_2 consumption is decreased).

In rats of normal weight the subcutaneous injection of the amino acid cystein (0.3 ml of a 5% solution per 100 g body weight) causes a significant increase in O_2 consumption in the course of one hour after injection (specific dynamic action). The same effect is observed in rat No. 79 in the initial period of increase in body weight, when it has not reached a high value (Table 4). In all the four remaining rats investigated with marked adiposity 4-11 months after injury to the region of the hypothalamus, no absorption of O_2 took place after the injection of cystein, i.e., the specific dynamic action of the amino acid was absent. The same result was observed by one of us (S. M. Leites) in mice with adiposity due to aurothioglucose.

It may be concluded from the findings as a whole that in rats with experimental hypothalamic adiposity the O_2 consumption in absolute figures is increased, but when calculated in terms of cm^2 body surface it is decreased. In the period of gain in weight the respiratory quotient is greater than unity; this is also observed in some animals after injection of glucose. This shows that the deposition of fat in hypothalamic adiposity is associated with an intensification of the conversion of carbohydrates into lipids, and in adipose rats this activation may take place also without hyperphagia. In severe hypothalamic adiposity the injection of cystein has no specific dynamic action, in contrast to its effect in animals of normal weight.

SUMMARY

In rats with experimental hypothalamic adiposity absolute O_2 consumption increases, but declines if taken as the rate per 1 cm^2 of the body surface. In the period of rising weight gain the respiratory quotient

exceeds 1; the same is observed in a number of animals after subcutaneous glucose injection. This indicates that deposition of fat in hypothalamic adiposity is connected with augmented conversion of carbohydrates into fats; this process may occur in adipose rats even without hyperphagia. Cystein administration does not exert any specific dynamic action in marked hypothalamic adiposity.

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ON THE RESORPTION OF PARENTERALLY ADMINISTERED SALINE SOLUTIONS IN EXPERIMENTAL SHOCK

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Since the parenteral method is widely used in antishock therapy to administer medical substances, a notion of how rapidly these substances enter the blood under such conditions, i.e., of the character of the resorption process in shock, is important. The resorption of substances introduced into tissue depends on the properties of these substances, the condition of the blood and lymph circulation in the tissues injected with the substances and, to some extent, the permeability of the blood and lymph capillaries.

As several experimental investigations have shown [3, 7, 8], the route by which the substances introduced into a healthy organism's tissue pass into the internal environment of the organism is determined to a specific degree by the properties of these substances. It has been established, for example, that substances of low molecular weight pass from the tissues into the blood [3, 7], while most substances of high molecular weight pass into the lymph [3, 7]. Malék and Kolc [5] have even discerned two types of resorption: the resorption of hematotropic substances and that of lymphotropic substances. They mention inulin and potassium thiocyanate as representatives of the first group and Congo red and dextran as representatives of the second.

According to their data, resorption in rabbits is dissociated under conditions of tourniquet shock: resorption through the blood capillaries is retarded, while resorption through the lymph capillaries is accelerated, and this change is directly proportional to the severity of shock. Resorption of hematotropic substances is retarded in traumatic shock, while that of lymphotropic substances is slightly accelerated, i.e., dissociation of the resorption processes occurs in this type of shock as well. Hemorrhagic shock is not attended by any pronounced dissociation of resorption.

It has also been observed that the passage of radiosodium from the tissues to the blood is retarded in shock, despite increased capillary permeability [1].

The literature does contain, then, a few works concerned with the resorption process under conditions of shock, but none of these have described how this process changes in different phases of experimental shock.

The subject of this investigation was the study of the process of resorption of parenterally administered substances in different phases of experimental shock.

EXPERIMENTAL METHOD

The experiments were performed on nonanesthetized cats. Shock was induced by A. M. Dubinskii's method: specific receptive areas of the animal's body were stimulated with a 300 picovolt pulsating current; the stimuli were 0.08 msec each in duration and were given at a frequency of four per second. Stimulation was applied through electrodes moistened with a physiological solution and bandaged onto the right paw. The shock phase was determined from the animal's general condition, the level and stability of the arterial pressure in the femoral artery, the pulse rate and the rate and character of the respiration. Comparison of these indices enabled us to distinguish four phases of shock: the erectile phase, torpid phases I and II and the terminal phase.

The rate of resorption from the tissues of microdispersed substances was determined according to:

1. The time required for semi-elimination of Na^{24} from the injection site [2, 4], which was determined as follows: 0.2 ml of a physiological solution containing 1 μc $Na^{24}Cl$ was injected intramuscularly, subcutaneously or intradermally with a fine needle; the rate per minute of the decomposition at the injection site was measured with an MST-17 end-window counter at the shortest possible time intervals.

2. The time at which the maximal concentration of P^{32} appeared in the circulating blood after the parenteral administration of the substance: 0.2 ml of a physiological solution containing 50 μc $Na_2HP^{32}O_4$ was injected as described above, after which 0.1 ml samples of peripheral blood were taken at specific time intervals, and their activity as expressed by the rate per minute of decomposition was measured with a type AC-2 Geiger counter.

EXPERIMENTAL RESULTS

The 37 control experiments established that the two methods of determining the resorption rate of parenterally administered substances give similar results. The most rapid resorption was observed when the experimental substances were intramuscularly administered (they were semiresorbed after 9-10 min); the resorption rate was slower with intradermal administration (20-21 min required for semiresorption) and slowest when the substances were subcutaneously administered (30-35 min required for semiresorption).

In 16 out of the 23 experiments with shock, we determined the resorption rate of substances injected into the adductor muscles of the right hind leg at different phases of shock; the other seven experiments studied the resorption rate of substances injected into the skin and subcutaneous cellular tissue of the same paw. The data obtained in these experiments, showing the average time required for semi-elimination of Na^{24} intramuscularly injected at different phases of shock and the average time required for the appearance of the maximal concentration of P^{32} (also administered intramuscularly) in the circulating blood, are given in the table.

Time Required for Semi-Elimination of Na^{24} and for the Appearance of the Maximal P^{32} Concentration in the Blood at Different Phases of Shock

Index of resorption rate	In control rabbits	In experimental rabbits at different phases of shock		
		erectile phase	torpid phase I	torpid phase II
Average time required for semi-elimination of Na^{24} from injection site (in min)	9.0	9.1	20.0	
Average time required for appearance of maximal P^{32} concentration in circulating blood (in min)	10.7	12.5	30.8	>33.0

From these data, it is evident that the time required for semi-elimination of intramuscularly injected Na^{24} and for the appearance of the maximal P^{32} concentration in the circulating blood steadily increased with each successive phase of shock, i.e., the rate of $Na^{24}Cl$ and $Na_2HP^{32}O_4$ resorption from the muscles became slower. For example, the slowing of the resorption phase was barely apparent in the erectile phase, while the resorption rate determined during torpid phase I was almost 2 ½-3 times slower than in the control.

Our data are in accord with the results obtained by Malék and Kolc [6] in their study of the resorption of certain hematotropic substances in rabbits with tourniquet shock.

During torpid phase II of shock, the resorption rate was still further retarded; the retardation was so great that semiresorption of the injected substance was not observed within the time range of the experiment. The resorption rate during the terminal phase was negligible.

This steady retardation of the resorption rate with each successive phase of shock was also observed in the experiments with the intradermal and subcutaneous administration of P^{32}. For example, one of the experiments in which the isotope solution was intradermally injected showed the time required for the appearance of the maximal P^{32} concentration in the circulating blood to be about 30 min in the erectile phase; in another experiment in which resorption was determined during torpid phase I, however, the time required for the appearance of maximal activity was more than $1\frac{1}{2}$ hours. As shock progressed to torpid phase II and the terminal phase, the resorption rate became so much slower that increase in the concentration of P^{32} following its intradermal administration was hardly noticeable.

The results obtained allow one to conclude that the resorption rate of parenterally administered microdispersed substances decreases under conditions of experimental shock, and that this retardation depends on the phase of shock, increasing with the severity of the latter.

SUMMARY

Parenteral administration of medicinal preparations during shock should be based on the knowledge of the resorption rate of medicinal substances in the tissues during various phases of shock. In this connection the resorption of $Na^{24}Cl$ and $Na_2HP^{32}O_4$ was studied after intramuscular, subcutaneous and intradermal injections. The rate of the resorption was assessed by the time required for semi-elimination of Na^{24} from the site of its administration, and for the appearance of the maximal P^{32} concentration in the circulating blood.

As established, the rate of resorption of parenterally administered microdispersed substances in experimental shock decreases. This delayed resorption is determined by the phase of shock and becomes greater with the increased severity of the latter.

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SPECIFIC FEATURES OF THE ANIMAL REACTION TO A SHOCK-INDUCING STIMULUS FOLLOWING THE ADMINISTRATION OF AMINAZINE*

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Neuroplegic substances of the phenothiazine series, especially Aminazine, have recently begun to be used to prevent and treat traumatic shock.

Laborit and Huguenard [5, 6] were the first to point out the value of Aminazine in traumatic and surgical shock. They believe, as do several other authors [1, 3], Aminazine's positive effect in shock to be due to its intensification of the processes of intrinsic inhibition and of inhibition in the zone of the reticular formation in particular.

Under experimental conditions, G. D. Chesnokova and B. A. Agaev [4] observed Aminazine to exert a favorable effect on animals in traumatic shock and then corroborated these data with clinical observations.

The purpose of this investigation was to further analyze Aminazine's effect on the course of traumatic shock. Since traumatic shock is a phasic process, we decided first to determine Aminazine's effect on the course and issue of shock changes depending on the phase in which the substance is administered.

Then, because Aminazine's hypotensive effect could be harmful in shock, we also determined the efficiency of its use in conjunction with vasopressor substances.

EXPERIMENTAL METHOD

The experiments were performed on nonanesthetized cats. Shock was induced by A. M. Dubinskii's method; the right paw of the animal was stimulated with a pulsating current four pulses per sec in frequency, 100 ma current force at the the height of each pulse and a stimulus duration of 0.08 msec. A kymograph was used to record the respiration and arterial pressure (in the femoral artery) throughout the experiment, the rectal temperature was taken and the general condition of the animal observed.

EXPERIMENTAL RESULTS

Thirty-five control experiments were performed in order to determine the characteristic course of the shock induced by the above method. These observations allowed us to distinguish three separate phases in the development of the shock: the erectile phase, torpid phase I and torpid phase II.

During the erectile phase of shock, we observed acute excitation of the animal, retarded respiration, a rise of arterial pressure to 180-200 mm of mercury and acceleration of the pulse. Torpid phase I was characterized by apathy, rapid, shallow respiration, the fall of arterial pressure and a decreased number of cardiac

* Chlorpromazine – Publisher.

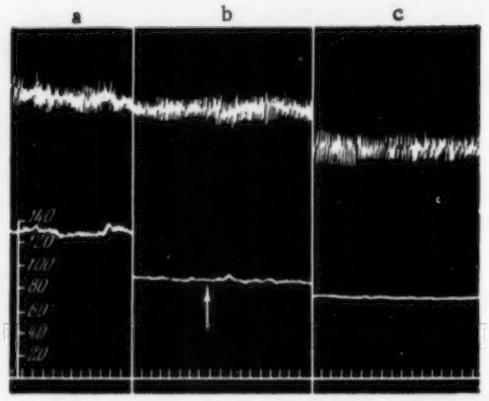

Fig. 1. Change in respiration and arterial pressure following administration of Aminazine during torpid phase I. 1) Original condition; b) during Aminazine administration (torpid phase I); c) one hour and 15 min after Aminazine administration. Curves show (from top to bottom): respiration; arterial pressure; time in 3-second marks. Arrow (↑) shows Aminazine administration.

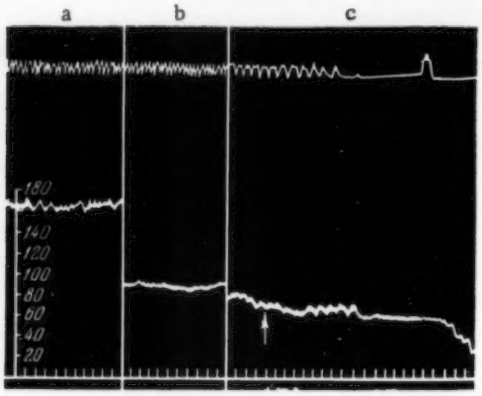

Fig. 2. Change in respiration and arterial pressure following Aminazine administration during torpid phase II. a) Original condition; b) torpid phase II; c) shock during Aminazine administration. Symbols the same as in Fig. 1.

contractions. Further retardation of the respiration, a steady fall of arterial pressure to 40-50 mm of mercury and retardation of the pulse were usually observed during torpid phase II.

We then studied the effect of Aminazine administered during the various phases on the course and issue of the shock. Aminazine was intramuscularly injected in a dose of 5 mg/kg on a background of the maximal arterial pressure. In the erectile phase of shock (7 experiments), a gradual fall of arterial pressure to 70-80 mm of mercury usually began 2-3 min after the Aminazine injection and then remained stable at the new level for a long time, despite continuing stimulation. There was not much change in the heart rate or that of the respiration. In these experiments, the more advanced phases of shock could only be induced by prolonged stimulation lasting 1-1½ hours, while only 30-40 min of stimulation were required to produce an analogous condition in the control experiments. Another difference from the control was that cessation of stimulation was followed by a stable rise of arterial pressure to 80-90 mm of mercury, even in cases when torpid phase II of shock was clinically apparent. The administration of Aminazine during the erectile phase rapidly diminished the animal's excitation and greatly reduced its response to extrinsic stimuli (touches, pricks).

The effect of Aminazine on the course of shock in torpid phase I was examined in 13 experiments.

In all the experiments, torpid phase I of shock was observed to be protracted and slow to change into torpid phase II. For example, torpid phase I of shock lasted an average of 25-30 min in the control experiments, but in the experiments with Aminazine, its duration stretched to 40-45 min, and more in some cases. After the administration of Aminazine, the arterial pressure decreased from the level usually characterizing torpid phase (90-100 mm of mercury) to 65-75 mm of mercury and then remained constant at the latter level. Slight acceleration of the respiration and pulse was observed (Fig. 1). The animals remained sluggish and apathetic both during the experiments and for quite a long while afterwards.

Torpid phase II of the shock was successfully developed in all 13 experiments by prolonging the action of the pulsating current on the animals; the arterial pressure, however, rose from 60 to 80-95 mm of mercury after stimulation ceased, which did not occur in the control animals.

The survival rate of the animals was also higher in the experiments; six out of the 13 cats given Aminazine during torpid phase I of shock and in which torpid phase II had clinically developed survived, while only one out of 15 animals survived in the analogous control experiments.

In five experiments, Aminazine was administered during torpid phase II of shock. In every case, the preparation was injected on a background of rather low arterial pressure (about 50 mm of mercury) and retarded pulse and respiration. After the Aminazine injection, further retardation of the respiration and heart rate was

usually observed along with a rapid fall of arterial pressure. The latter fell from 50-40 to 20-10, often even to 0 mm of mercury within 2-5 min, and the animal died (Fig. 2).

Aminazine, therefore, alters the course of shock and makes animals more resistant to a shock-inducing stimulus when administered during the erectile phase or torpid phase I of shock. However, Aminazine is known to possess a pronounced hypotensive effect, which considerably limits its use in shock. To prevent the development of hypotonia after the administration of Aminazine, B. I. Lyubimov [2] recommends that it be administered in conjunction with either Pituitrin or noradrenalin.

In a previous work, we established that both noradrenalin and Pituitrin, when administered under conditions of experimental shock, induce a rise of arterial pressure and its stabilization at the new level; Pituitrin has the more lasting effect of the two. On this basis, we used Pituitrin in this work to prevent Aminazine-induced hypotonia.

In the experiments conducted to determine the effect of the Aminazine-Pituitrin combination on the course of shock, Aminazine was intramuscularly injected in a dose of 5 mg/kg, as in the previous experiments, and then, as soon as the arterial pressure showed a tendency to fall, 1 ml Pituitrin containing 3 units was administered.

In five experiments, Aminazine and Pituitrin were administered in torpid phase I of shock. In every case, the arterial pressure rose 20-30 mm of mercury a few minutes after the administration of Pituitrin and was restored to the initial level within 10-15 minutes despite continuous application of the stimulation. It was interesting that the respiration and heart rate hardly changed at all during these experiments. All five animals of this group survived despite prolonged application of the stimulation and the fact that the shock reached a depth corresponding to torpid phase II.

In the next five experiments, Aminazine and Pituitrin were administered during torpid phase II of shock, and the acute fall of arterial pressure observed when Aminazine was administered alone during this phase of shock did not occur in any of these experiments.

In three experiments of this series, the administration of Pituitrin increased the severity of the shock, and the animals died 40-50 min afterwards; one animal died later than this, but within the first 24 hours after the experiment, and one animal survived.

The data presented demonstrate that Aminazine used in conjunction with Pituitrin during torpid phase I and even torpid phase II of experimental shock has a positive effect on the course and issue of the shock. G. D. Chesnokova and B. A. Agaev [4] observed, as we did, Aminazine to exert a favorable therapeutic effect when administered at early stages of shock; when it was administered during stage III (analogous to our torpid phase II), however, the death of the animals was expedited. In order to ensure the maintenance of a sufficiently high arterial pressure level, they proposed the use of blood transfusion before the administration of Aminazine. It is hardly expedient to increase the total blood volume, however, in shock cases,where there is no blood loss and there is a decrease in the volume of the circulating blood due to the development of capillary stasis. In such cases, we believe it more effective to limit dystonia of the peripheral vessels by the use of vasopressor substances with a prolonged effect, such as Pituitrin, for example, which we used in our experiments.

SUMMARY

The author studied peculiarities attending the course of experimental shock with Aminazine (largactyl) administered during its various phases (erectile, the I and the II torpid). Aminazine therapy proved to be the most effective during the erectile and the I torpid phases, whereas its use during the II torpid phase leads to rapid death of the animals.

The hypotensive properties of Aminazine limit the possibilities of its application in shock. Therefore, it is best to administer it in conjunction with vasopressor substances. Combined Aminazine – Pituitrin administration provokes a positive therapeutic effect on the course and the outcome of an experimental shock.

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THE INFLUENCE OF THE FUNCTIONAL CONDITION OF THE CENTRAL NERVOUS SYSTEM ON THE DEVELOPMENT OF TOXIC EDEMA OF THE LUNGS AND PLEURISY

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The genesis of toxic edema of the lungs and pleurisy, among other diseases, is not yet sufficiently understood. Several researchers believe the pathogenesis of these pathologic processes to be based on injury to the nervous system [2-6].

The purpose of this work was to find new evidence supporting and elaborating the neurogenic theory of the pathogenesis of pulmonary edema and pleurisy. Our aim was to determine the influence of the functional condition of the central nervous system and change in the relative strength of the nervous excitation-inhibition processes on the development of toxic pulmonary edema and pleurisy.

EXPERIMENTAL METHOD

The investigations were conducted on dogs and cats. Pharmacological substances were used to alter the functional condition of the central nervous system: somnifacients (Bromural, Adalin and Medinal), sodium bromide, caffeine and Phenamine. Acute pulmonary edema and pleurisy were induced by the administration of a sodium iodide solution.

The experimental animals were first given one of the above pharmacological substances, perorally administered, and then an intravenous injection of sodium iodide in a dilution of 1:2, or, sometimes, 1:4. We conducted parallel observations on control animals which were intravenously injected with sodium iodide in the same doses and dilutions used for the experimental animals.

To define the degree of pulmonary edema and pleurisy, we observed the condition of the animals, made macroscopic and microscopic studies of lung preparations* and determined the changes in the pleural cavities. We also determined the weight of the lungs and the amount of fluid in the pleural cavities. The blood was examined in several experiments to determine the hemoglobin content and number of erythrocytes.

EXPERIMENTAL RESULTS

In the first series of experiments, we studied the effect of somnifacient substances and sodium bromide on the development of pulmonary edema and pleurisy induced by the administration of sodium iodide. One hundred and forty-four dogs and ten cats were used in the experiments with somnifacient substances. The effect of sodium bromide was tested on 24 dogs.

* The histological preparations were prepared by the usual method. Pieces of lung were passed through alcohols of varying strengths and xylene, then embedded in paraffin. The sections were stained with hematoxylin-eosin and with van Gieson's stain.

TABLE 1

Results of the Experiments with Somnifacient Substances, Sodium Bromide and Sodium Iodide

Experiment				Control			
Number of animals	Animals received per 1 kg weight	Average weight of lungs (in g per kg animal weight)	Number of pleurisy cases	Number of animals	Animals received per 1 kg weight	Average weight of lungs (in g per kg animal weight)	Number of pleurisy cases
20	0.6 g Bromural and 2 ml NaI	18.1	2	10	2 ml NaI	25.9	5
12*	0.5 and 0.6 g Adalin and 2 ml NaI	20.1	3	12	2 ml NaI; stimulation of vagosympathetic trunk and 2 and 3 ml NaI	27.2	7
40	0.6 g Bromural; stimulation of vagosympathetic trunk and 2 and 3 ml NaI	19.5	6	40		24.7	25
6	0.01 sodium bromide and 0.5 ml NaI	10.8	0	6	0.5 ml NaI	20.2	3
6	0.1 g sodium bromide and 0.5 and 1 ml NaI	18.5	3	6	0.5 and 1 ml NaI	15.3	2

* Two of the dogs (one experimental and one control) did not die.

Bromural and Adalin were administered to 32 dogs. The sodium iodide solution was injected as soon as sleep or narcosis developed. The 22 control dogs received only the sodium iodide solution. Autopsies were performed on all the animals which died (Table 1).

Table 1 shows that the average weight of the lungs and the incidence of pleurisy was less in the animals which had received Bromural or Adalin than in the control animals. Macroscopic and microscopic examinations of the lungs confirmed these data (Figs. 1, 2). The blood change (pyknosis) was greater in the control animals.

Analogous results were obtained in the experiments with cats given Medinal in a dose of 0.5 g/kg weight before being injected with sodium iodide.

In the doses administered, therefore, the somnifacients Bromural and Adalin in the dogs and Medinal in the cats inhibited the development of pulmonary edema and prevented pleurisy.

These data prompted us to determine the effect of somnifacients on the development of toxic edema of the lungs and pleurisy against a background of change in the functional condition of the central nervous system.

Experiments conducted earlier in collaboration with K. F. Berkgaut [1] demonstrated that stimulation of the vagosympathetic trunk to the neck with an ammonium hydroxide solution promotes the development of pleurisy and pulmonary edema in dogs poisoned with sodium iodide. In this present work, we performed experiments in which Bromural was administered before and after stimulation of the vagosympathetic trunk and injection of the sodium iodide solution.

Bromural was administered to 40 dogs. After sleep or narcosis had developed, the animals were given a centrally directed injection in the right vagosympathetic trunk of a 50% ammonium hydroxide solution in a dose of 0.1-0.2 ml. Then the sodium iodide solution was administered to the dogs. Stimulation of the vagosympathetic trunk was done the same way in the control dogs (40), before the sodium iodide solution was administered. Autopsies were performed after the death or sacrifice of the animals.

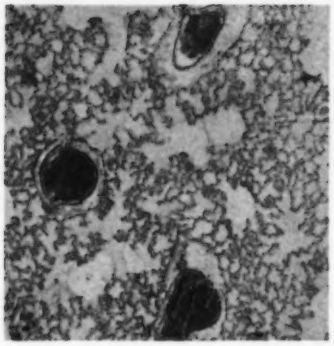

Fig. 1. Change in dog's lung tissue following administration of Bromural and injection of sodium iodide solution. Plethora. Slight perivascular edema. Magnification: ocular 1 ×, objective AA. Stained with hematoxylin-eosin.

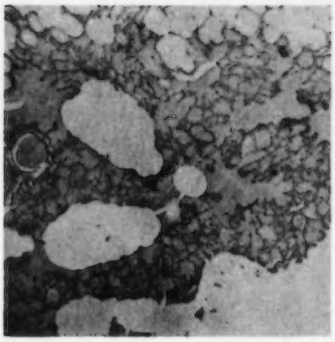

Fig. 2. Change in dog's lung tissue following injection of sodium iodide solution. Alveolar edema. Atelectasis and emphysema. Magnification: ocular 1 ×, objective AA. Stained with hematoxylin-eosin.

The results shown in Table 1 indicate that the average weight of the lungs and the incidence of pleurisy was less in the experimental than in the control animals. The autopsies disclosed stasis and edema in the lungs of only a few experimental animals, while these changes were more pronounced and consistently observed in the control animals. The control animals also showed greater pyknosis of the blood.

The investigations conducted on 10 dogs to which Bromural was administered after stimulation of the vagosympathetic trunk and the injection of sodium iodide showed that, in a dose of 0.5 g/kg animal weight, the preparation did not affect the development of pulmonary edema and pleurisy. In a dose of 0.7 g/kg weight, however, Bromural prevented the development of these processes.

Therefore, under conditions of stimulation of the vagosympathetic trunk, the administration of Bromural did affect the development of pulmonary edema and pleurisy in dogs poisoned with sodium iodide.

By inducing inhibition in the central nervous system, then, somnifacients change its functional condition and thereby prevent the development of edema of the lungs and pleurisy.

On the basis of these data, we set up experiments with sodium bromide, a substance which induces concentrated inhibition in the central nervous system.

Sodium bromide was administered in "small" (0.01 g/kg) or "large" (0.1 g/kg) doses to twelve dogs (Table 1). Then the sodium iodide solution was injected when the dogs were asleep or in a somnolent condition. The control animals received only the sodium iodide solution. Autopsies were performed after the death or sacrifice of the animals.

As Table 1 shows, no pleurisy was found in the animals which had received the "small" dose of sodium bromide, and the average weight of the lungs was within the normal range. Pleurisy was observed in the control animals, and the average weight of the lungs considerably exceeded the normal. The macroscopic and microscopic examinations of the lungs showed no edema in any of the experimental animals. Considerable edema of the lung tissue was observed in the control animals.

In the animals which had received the "large"dose of sodium bromide, pleurisy was found more frequently and the average weight of the lungs was considerably more than in the control. The macroscopic and microscopic examinations of the lungs showed a greater degree of pulmonary edema in 3 of the experimental animals than in any of the control, while the pulmonary edema found in the other 3 animals was analogous to that observed in the control.

TABLE 2

Results of the Experiments with Phenamine, Caffeine and Sodium Iodide

Experiment				Control			
Number of animals	Animals received per 1 kg weight	Average weight of lungs (in g per kg animal weight)	Number of pleurisy cases	Number of animals	Animals received per 1 kg weight	Average weight of lungs (in g per kg animal weight)	Number of pleurisy cases
15	0.0003 and 0.0008 g Phenamine and 0.5, 0.75, 1, 1,5 and 2 ml NaI	20.7	8	15	0.5, 0.71, 1, 1,5 and 2 ml NaI	28.4	13
15*	0.001, 0.002 and 0.005 g Phenamine and 1, 2 and 3 ml NaI	25.9	12	15	1, 2 and 3 ml NaI	19.6	6
4	0.0015 g caffeine and 0.75 and 1 ml NaI	27.4	4	4	0.75 and 1 ml NaI	31.7	4
6	0.01 g caffeine and 0.75 ml NaI	22.9	4	6	0.75 ml NaI	13.5	1
6	0.1 g caffeine and 0.75 ml NaI	14.3	1	6	0.75 ml NaI	21.0	3

* One of the experimental and 3 of the control dogs did not die.

The "small" dose of sodium bromide, therefore, prevented the development of pulmonary edema and pleurisy in the animals following the administration of sodium iodide, while the "large" dose either had no effect on these processes or intensified them.

Consequently, the development of pulmonary edema and pleurisy in dogs poisoned with sodium iodide is affected by the inhibition induced by the administration of sodium bromide as well as by that induced by the administration of somnifacients.

A second series of experiments was conducted to determine the effect of Phenamine and caffeine on the development of pulmonary edema and pleurisy induced by the administration of sodium iodide.

Phenamine was administered in "small" (0.0003 and 0.0008 g/kg) or "large" (0.001, 0.002 and 0.005 g/kg) doses to 30 dogs. Then the sodium iodide solution was injected. Autopsies were performed after the death or sacrifice of the animals.

The data in Table 2 show that the incidence of pleurisy and the average weight of the lungs were less in the animals which had received Phenamine in "small" doses than in the control animals. Macroscopic and microscopic examinations of the lungs disclosed pulmonary edema in some of the experimental and all of the control animals.

In the "large" doses, Phenamine promoted the development of pleurisy and edema of the lungs, causing the weight of the latter to increase. Alveolar edema, a typical change induced in the lungs by the action of the "large" doses of Phenamine, was less pronounced in the control animals.

Therefore, the development of pulmonary edema and pleurisy in dogs poisoned with sodium iodide is inhibited by "small" doses of Phenamine, but intensified by "large" doses of this substance.

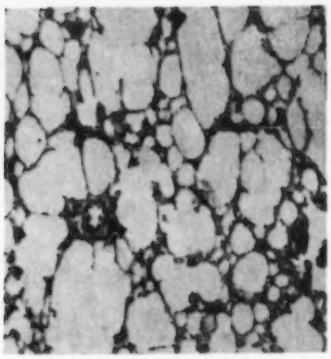

Fig. 3. Normal lung tissue of a dog after the administration of a "large" dose of caffeine and the injection of the sodium iodide solution. Magnification: ocular 8 ×; objective 10 ×. Stained with hematoxylin-eosin.

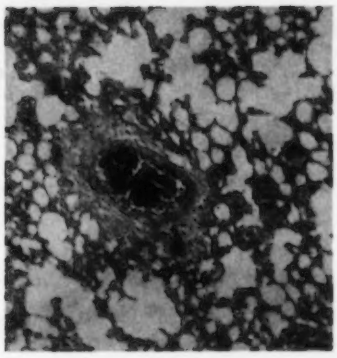

Fig. 4. Lung tissue of a dog after the administration of the sodium iodide solution. Stasis. Perivascular edema. Magnification: ocular 8 ×; objective 10 ×. Stained with hematoxylin-eosin.

Experiments with caffeine were performed in which the preparation was administered in "small" (0.0015 g/kg), "medium" (0.01 g/kg) and "large" (0.1 g/kg) doses to 16 dogs before the injection of sodium iodide. Autopsies were performed after the death or sacrifice of the animals. The results are given in Table 2.

Table 2 shows that the "small" dose of caffeine inhibited the development of edema of the lungs, the average weight of which was less than in the control, but did not affect the development of pleurisy. Macroscopic and microscopic examinations of the lungs showed pulmonary edema to be less pronounced in the experimental animals than in the control.

In the "medium" dose, caffeine intensified pulmonary edema and promoted the development of pleurisy. The macroscopic and microscopic investigations showed the changes in the lungs of the experimental animals to be much greater than in those of the control.

The "large" dose of caffeine inhibited the development of pulmonary edema and pleurisy in dogs poisoned with sodium iodide. This was confirmed by the microscopic findings (Figs. 3 and 4).

In the "medium" dose, therefore, caffeine intensifies the development of both pulmonary edema and pleurisy, in the "small" dose, inhibits pulmonary edema but does not affect the development of pleurisy and in the "large" dose, inhibits the development of both these changes induced by the administration of sodium iodide.

The results of our experimental study of pulmonary edema and pleurisy can be summarized by the statement that change in the functional condition of the central nervous system affects the development of these processes. The balance between the principal nervous processes, excitation and inhibition, is particularly significant in this connection. The experiments have shown that therapeutic substances which influence these processes can by so doing inhibit or promote the development of pulmonary edema and pleurisy.

This is further confirmed by the results of the experiments in which the vagosympathetic trunk was stimulated before and after the development of Bromural-induced inhibition in dogs poisoned with sodium iodide.

Therefore, regulation of the balance between the inhibition and excitation processes in the central nervous system can play an important part in the prevention of pulmonary edema and pleurisy. Clinicians treating pulmonary edema and pleurisy should, then, take into account the role which we have shown the central nervous system to play in the development of these diseases.

SUMMARY

The author studied the effect of the functional condition of the central nervous system and especially of the changes in the correlation of the main nervous processes – excitation and inhibition – on the development

of toxic edema of the lungs and pleurisy. The changes in the functional state of the central nervous system were produced by the administration of sodium bromide, caffeine, and Phenamine (Benzedrine); stimulation of the vagosympathetic trunk was also used. Acute edema of the lungs and pleurisy were induced by sodium bromide administration. The results of experiments have demonstrated that alteration of the functional condition of the nervous system, particularly of the correlation of the excitation-inhibition processes, had a definite influence on the development of toxic edema of the lungs and pleurisy.

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CHANGE IN THE SEROTONIN CONTENT OF THE BLOOD OF RABBITS FOLLOWING TYPHOID INTOXICATION

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Researchers have recently shown increasing interest in the study of the physiological and pharmacological properties of the very active biogenous compound serotonin (5-hydroxytryptamine, 5-HTA), as well as in explanation of its role in various pathologic conditions [3, 19, 31, 35]. M. O. Raushenbakh and G. A. Chernov [2], for example, believe 5-HTA to be very important in the pathogenesis of acute radiation sickness; 5-HTA possesses a histamine-like effect and is evidently one of the phlogogenic substances which play a large part in the pathogenesis of infectious diseases. Shimamoto and co-workers [41,42] believe 5-HTA to be a toxicity factor in dysenteric intoxication. These [40, 41, 42] and other [16, 25] authors have established that bacterial endotoxins cause the liberation of 5-HTA from thrombocytes. The lethality of the dysenteric endotoxin has been reduced by the preliminary administration of 5-HTA antagonists [40] or reserpine [44] to animals. The administration of 5-HTA to mice increases their sensitivity to the causative agent of whooping cough [24, 28, 33]. Erspamer [20], however, believes that 5-HTA is a histogenous hormone which is active at its formation site, and considers the proposal that surplus 5-HTA plays a part in the genesis of various diseases to be poorly substantiated.

We therefore decided to determine how the serotonin content of the blood and of organs with a fairly high 5-HTA content changes during the development of bacterial intoxication.

The work was carried out on 42 male rabbits weighing 2.5-3 kg each. The animals were intravenously injected with heat-killed typhoid vaccine, prepared from the laboratory strain No. 495 of E. typhosa, in a dose of 30 billion microbes per 1 kg body weight. Determination of 5-HTA in the blood was carried out before the vaccine was administered, and then 5-HTA was determined in the blood and organs 15 and 30 min, 1, 3, 5, 12 and 24 hr after the administration of the vaccine. Two or three ml of blood was taken from the marginal vein of the rabbit's ear or from the heart; the rabbits were then exsanguinated by transecting the neck vessel under the light ether anesthesia, after which the brain, lungs, the last 50 cm of the small intestine and a 5 × 5 cm skin flap from the middle of the right side were immediately removed from the animals. As the control, we used the same organs, taken from rabbits which had not received the vaccine. Homogenates of the experimental organs were prepared. The serotonin content per 1 g each organ's homogenate and per 1.5 ml blood was determined. We used 0.1 ml of a 3.8% sodium citrate solution per 1 ml blood to prevent clotting. The method of Udenfriend and co-workers [46] was used to extract 5-HTA from the blood and organs. Quantitative determination of 5-HTA was done by the biological method, using a rat's isolated colon [15]. Gaddum's fluid was used to nourish the isolated colon and to dilute the serotonin extracts. We used the Flyuk Company's (Italy) creatinine sulfate 5-HTA as the standard.

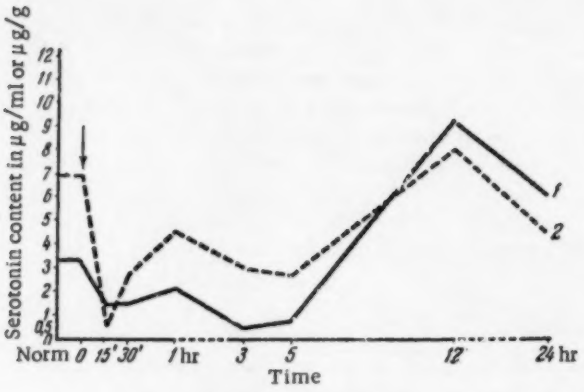

Fig. 1. Change in serotonin content of rabbits' blood and lungs following typhoid intoxication. 1) Blood; 2) lungs; ↓) administration of typhoid vaccine.

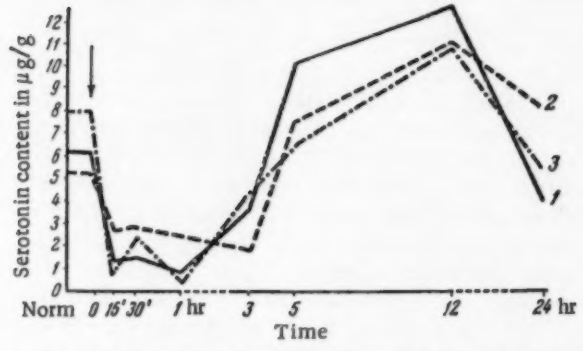

Fig. 2. Change in serotonin content of brain, small intestine and skin of rabbits following typhoid intoxication. 1) Brain; 2) small intestine; 3) skin; ↓) administration of typhoid vaccine.

The average data of the results obtained are shown in Figs. 1 and 2. Figure 1 shows that the 5-HTA content of the blood undergoes phasic changes. A sharp decrease in the 5-HTA content of the rabbit's blood was observed 15 min after the administration of the typhoid vaccine. The 5-HTA content decreased to 2.3 times less than the original level (from 3.28 ± 0.47 μg/ml to 1.44 ± 0.24 μg/ml). The content of 5-HTA in the blood remained below the original level for a long time. Three to five hr after the typhoid vaccine was administered, the 5-HTA content was 1/8 – 1/5 the original. Twelve hours after the vaccine injection, when the animals' condition had considerably deteriorated and severe diarrhea had developed, the 5-HTA content of the blood increased sharply, to thrice the original level. After 24 hr the 5-HTA content began to decrease, but its level during this period was still twice as high as the original. The animals' death prevented further determination of the 5-HTA content of the blood at later periods.

The changes observed in the serotonin content of the lungs, brain, small intestine and skin were also phasic in nature. The curve showing the 5-HTA content of the lungs repeated the curve of the 5-HTA content of the blood (see Fig. 1). Figure 2 shows the data characterizing the changes in the 5-HTA content of the brain, small intestine and skin. Fifteen minutes after the vaccine injection, a sharp decrease in the serotonin content of these organs was observed. For example, the average 5-HTA content of the small intestine decreased to $\frac{1}{2}$ the original level, while in the brain and skin, the average 5-HTA content decreased to, respectively, $\frac{1}{4}$ and $\frac{1}{5}$ the original levels. The 5-HTA content continued to be rather low for an hour in the skin and brain and for three hr in the small intestine.

If the curves showing the 5-HTA content of the brain, skin and small intestine are compared with the curve of its content in the blood, it is evident that the 5-HTA of the organs undergoes the same changes during the initial period of the intoxication as the 5-HTA of the blood. The subsequent increase in 5-HTA content begins considerably earlier in the case of the organs than in that of the blood. Five hr after the administration of the typhoid vaccine, when the 5-HTA content of the blood was still very low, the 5-HTA content of the rabbits' brain and small intestine was higher than in the same organs of the control rabbits. Twelve hr after the typhoid vaccine injection, these organs contained twice as much 5-HTA as the corresponding organs of the control rabbits. In the experimental rabbits during this period, the 5-HTA content of the skin was also higher than the amount found in the skin of the control animals. After 24 hr , the 5-HTA content of the experimental organs decreased. The 5-HTA decrease was especially sharp in the brain, skin and lungs; in these organs, its content became considerably lower than in the corresponding organs of the control rabbits. In the intestine, the 5-HTA content remained fairly high, exceeding the control level. During this period, the condition of the rabbits was very serious. Adynamia, diarrhea and lack of reaction to extraneous stimuli were observed in the animals.

As a result of the observations conducted, several reasons have been established for the phasic nature of the changes in the 5-HTA of the blood following typhoid intoxication. During the first hr of typhoid intoxication, the change in the 5-HTA content of the blood can be related to change in the number of thrombocytes; subsequently, no direct relationship between these indices is observed. M. O. Raushenbakh and G. A. Chernov were also unable to observe a constant relationship between the 5-HTA content of the blood and the number of thrombocytes in different animals with acute radiation sickness [2]. The most important reasons seem to be the liberation of 5-HTA from the thrombocytes effected by the typhoid endotoxin and change in the adsorptive properties of the thrombocytes. There are reports in the literature that bacterial endotoxins prepared from Shigella sonnei [40, 41] and Clostridium Welchii [25] cause the liberation of 5-HTA from the thrombocytes. It is well known that 5-HTA is not synthesized in thrombocytes, but enters these cells by adsorption [11, 52] and that the influence of unfavorable factors causes the adsorptive ability of thrombocytes to decrease [2, 11, 26].

Change in the 5-HTA content of the blood and organs is undoubtedly affected by the processes of its formation, which depends on the activity of the enzyme 5-hydroxytryptophan carboxylase [9, 29, 38, 49], and decomposition, which depends on the activity of the enzyme monamine oxidase, which deaminates 5-HTA, forming hydroxyindoleacetic acid and 5-hydroxyindoleacetic acid [7, 9, 32, 47, 48]. There is every reason to believe that the activity of the latter enzyme changes during the development of typhoid intoxidation, as certain authors have observed it to following the irradiation of animals [1].

The sharp increase in the 5-HTA content of the small intestine and blood 5-12 hr after the administration of the typhoid vaccine could be the cause of the diarrhea observed in the rabbits during this period. There are many reports in the literature that an increase in the 5-HTA content of the organism intensifies intestinal peristalsis and causes the occurrence of diarrhea [10, 12, 21, 30].

The indirect results of our study indicate that serotonin is evidently formed or bound in the small intestine, brain and skin of the rabbit, creating depots in certain cells of these organs. In the literature, it has been shown that the argentaffine cells of the gastrointestinal tract mucosa contain a large amount of 5-HTA [5, 17, 18] and that in these cells, tryptophan is transformed into 5-hydroxytryptophan and from that into 5-HTA [18]. Several authors [9, 34, 50] believe that 5-HTA is formed in certain organs of the central nervous system. In the skin of rats and mice, the fat cells are a source of 5-HTA [27, 29, 37, 54], but in other animals and in man, the serotonin in the skin is not associated with the fat cells [6, 22, 39, 43, 44, 53, 54], but is probably bound by other structural elements. No accumulation of 5-HTA in the lungs has been established; therefore, the great similarity of the changes we observed in the 5-HTA content of the blood and lungs following typhoid intoxication (see Fig. 1) suggests that the 5-HTA content of the lungs depends to a large extent on the 5-HTA content of the blood. Certain authors, however, oppose this point of view [23, 26].

SUMMARY

The content of serotonin in the blood, brain, small intestine, lungs and skin undergoes phasic changes during the process of typhoid intoxication in rabbits. The amount of serotonin in the blood and organs drops markedly 15 min after intravenous injection of typhoid vaccine and remains below the initial level for a long period of time. The rise of serotonin level in the organs begins earlier than in the blood. In 12 hours after the

administration of the vaccine, serotonin concentration considerably exceeds its initial level in the blood and organs. Repeated reduction of serotonin content is seen in 24 hr.

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THE ANTIPYRETIC EFFECT OF PYRAMIDON IN VACCINAL FEVER, DINITROPHENOL HYPERTHERMIA AND EXPERIMENTAL DIPHTHERIA INTOXICATION

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The present theory of the reasons for the temperature rise in fever is based on the notion of reactive change in the functional condition of the thermoregulator centers. According to this theory, not every rise of temperature (not associated with external overheating) can be regarded as fever.

For example, the physiological nature of a temperature rise resulting from an increase in thermogenesis so extreme that its compensation exceeds the adaptive powers of thermoregulation has nothing in common with that of the fever reaction. The hyperthermia induced by the administration of 2,4-dinitrophenol (DNP) is a typical experimental example of such a "metabolic" temperature rise.

The main difference in the condition of the thermoregulator apparatus in dinitrophenol hyperthermia and in fever lies in the fact that although the mechanisms of heat emission are strained to the maximum in the former, the increase in thermogenesis is still greater. When the heat emission of the organism is increased by external cooling, hypothermia does not develop [1, 2]. Hyperthermia, however, increases very rapidly when the temperature of the external environment is moderately high [5, 6].

The temperature conditions of the external environment have little influence on the temperature rise in fever.

In order to further characterize the physiological mechanisms of these two conditions, we decided to compare the antipyretic effects of Pyramidon in fever and in DNP-induced hyperthermia.

Reports that the thermoregulator apparatus is disturbed in diphtheria intoxication [5, 6] led us to investigate in addition Pyramidon's effect on the fever attending this intoxication, a question of particular value in analyzing the condition of thermoregulation.

EXPERIMENTAL RESULTS

The experiments were performed on rabbits weighing 2500-2800 g each. The antipyretic effect of Pyramidon on hyperthermia induced by DNP was studied in the first series of experiments, performed on 14 rabbits.

The experiments were conducted as follows: after the administration of DNP (in doses of 15-30 mg/kg) to a rabbit, the changes in its rectal temperature were observed for 6 hr. A few days later, the same rabbit was given a subcutaneous injection of 0,1 g/kg Pyramidon in the form of a 2% solution.

Ten days or more after these control experiments, we performed experiments in which Pyramidon was administered on a background of hyperthermia.

In the control experiments, 30 mg/kg DNP caused a temperature rise of 0.4-0.8° within 30 min. The maximal temperature rise was observed after 2-3 hr (1.9-3.3°), after which the temperature began to decrease. Six hours after the administration of the drug, the temperature became stabilized at a level 0.4-1° higher than the original (Fig. 1a).*

The administration of Pyramidon to healthy animals caused a temperature drop of 1.3-1.8°, the maximal drop being observed after 1-1½ hr; the temperature than began to rise and usually reached the original level in 4-5 hr (Fig. 1b).

When Pyramidon was administered 45 min after the DNP injection (i.e., when the temperature had risen 0.6-0.8°), it did not either reduce the temperature or inhibit its rise. In these cases, the ultimate temperature rise equalled or even exceeded that observed with the administration of DNP alone (Fig. 1c).

The temperature rise caused by the administration of 20 mg/kg DNP (0.5-1.4°) and the time required for the temperature to return to its original level were much less than with the 30 mg/kg dose. However, no reduction of temperature was effected by the administration of Pyramidon after this dose of dinitrophenol either.

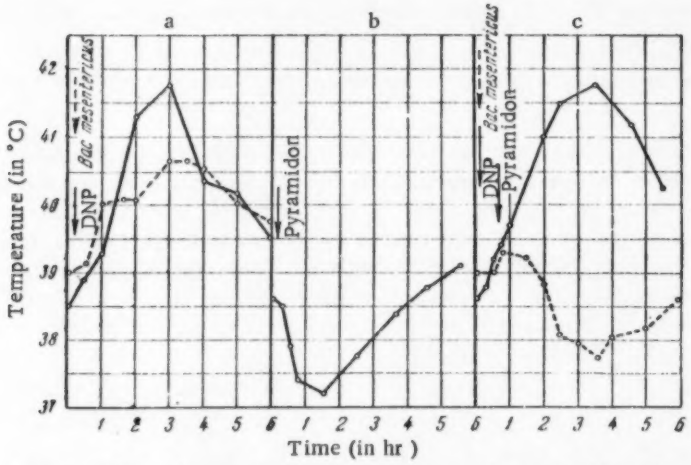

Fig. 1. Effect of Pyramidon on the temperature of normal rabbits given large doses of DNP and in vaccinal fever. a) Temperature curves after administration of 0.03 g/kg DNP (solid line) and 1 ml/kg Bac. mesentericus vaccine (dotted line). Solid arrow – DNP administration; dotted arrow – vaccine administration; b) temperature curve of control animal after administration of 0.1 g/kg Pyramidon (control); c) temperature curves resulting from administration of 0.1 g/kg Pyramidon (thin solid arrow) to rabbits 45 min after injection of 0.03 g/kg DNP (thick solid arrow and solid curve) and one hour after injection of Bac. mesentericus vaccine (dotted arrow and curve).

The 15 mg/kg dose of DNP caused only a slight rise of temperature (0.3-0.7°), the maximum being observed within 2-3 hr (Fig. 2a). As in the preceding experiments, Pyramidon was administered 45 min after the DNP injection, when the temperature rise constituted 0.1-0.3°. The temperature was down to its original level one hour after the Pyramidon injection; the maximal fall (0.5-1.3°), observed 2 hr after the injection, was less than in the control experiments (Fig. 2b). The temperature returned to its original level about 5 hr after the injection (Fig. 2c).

* This and the other figures show individual curves typical for each series of experiments.

A second series of experiments was conducted on eight rabbits in order to determine Pyramidon's antipyretic effect on fever induced by the injection of 1 ml/kg of a Bac. mesentericus culture into the vein of the ear. Our previous investigations have described Pyramidon's effect on this type of experimental fever in rabbits in greater detail.

The culture injection induced a rise of temperature in 30 min. The maximal temperature rise (1-1.5°) was observed after 1½-2 hrs, the original temperature being restored about 6-7 hr after the injection.

A 0.1 g/kg dose of Pyramidon in a 2% solution was administered either 30 min before administration of the pyrogenic vaccine or at the height of the fever. When injected 30 min before the Bac. mesentericus injection, Pyramidon not only prevented the development of the fever reaction, but actually reduced the temperature to 1.1-2.5° below the original level.

When Pyramidon was administered after the injection of the pyrogenic culture, the temperature usually fell 1.5-2° below the original level (see Fig. 1c).

A third series of experiments was conducted on 31 rabbits to study the antipyretic effect of Pyramidon in diphtheria intoxication. The toxin was used in a dilution of 1:100 and injected in a dose of a 3 MLD per animal. The disease was pronounced in the rabbits on the 1st and 2nd days following the administration of the diphtheria toxin, which was clinically apparent from the loss of weight, appetite and muscular tonus and the acute rise of temperature.

Pyramidon was injected daily, either starting the day the toxin was administered or starting the third day of the disease.

When Pyramidon was administered one hour after the toxin was injected, the rabbit's temperature fell to about the same level as in the control experiments (Fig. 3a), returning to the original level in 5-6 hr. When Pyramidon was administered the following day to the same rabbit, the temperature was reduced even more and was still low after 5-6 hr (Fig. 3b). When Pyramidon was again administered on the third day of the disease, when the rabbit's temperature was still low (36.4°), the temperature dropped sharply and showed no tendency to rise even after 6 hr.

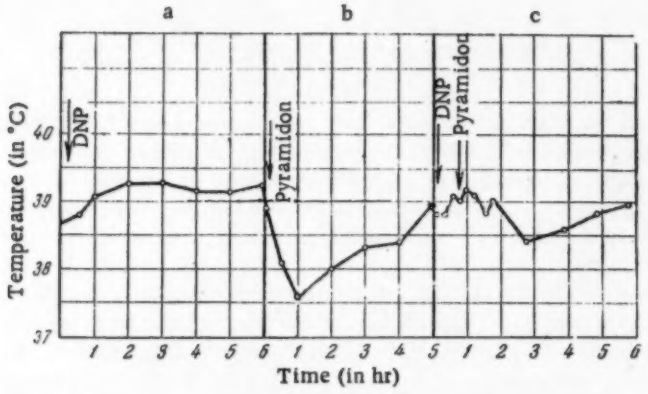

Fig. 2. Change in temperature following administration of Pyramidon to rabbits which had received a small dose of DNP. a) Injection of 0.015 g/kg DNP; b) administration of 0.1 g/kg Pyramidon to control rabbit; c) 0.1 g/kg Pyramidon injected 45 min after injection of 0.015 g/kg DNP.

When Pyramidon was injected for the first time on the third day of the disease, the temperature dropped and remained low until the death of the animal.

The experiments conducted demonstrated that Pyramidon has no effect on dinitrophenol hyperthermia, although it has a sharply pronounced antipyretic effect on both vaccinal and toxic fever. Only with the

administration of a small dose of DNP, 15 mg/kg, the threshold dose required for the development of hyperthermia, was a slight effect observed. The failure of Pyramidon to exert its antipyretic effect with the administration of large doses of DNP is evidently because the thermoregulator apparatus, which was functioning at full capacity even before the administration of the antipyretic, was unable to further increase heat emission.

The data obtained, therefore, show that the temperature rise which follows DNP administration is not due to active reorganization of the thermoregulator apparatus, as is true in the case of fever, but rather to the insufficiency of the adaptive faculties of the thermoregulator apparatus which cannot provide adequate heat emission to balance the excessive heat production induced by the administration of DNP. The temperature drop caused by the administration of Pyramidon in an animal with fever is known to be primarily due to "forced" enhancement of the heat emission processes.

Therefore, the temperature drop induced by Pyramidon in animals injected with the Bac. mesentericus culture or diphtheria toxin and its failure to exert an antipyretic effect in animals which have received DNP are further proof that the mechanism of temperature rise and the functional condition of the thermoregulator center are physiologically different in fever and in dinitrophenol hyperthermia.

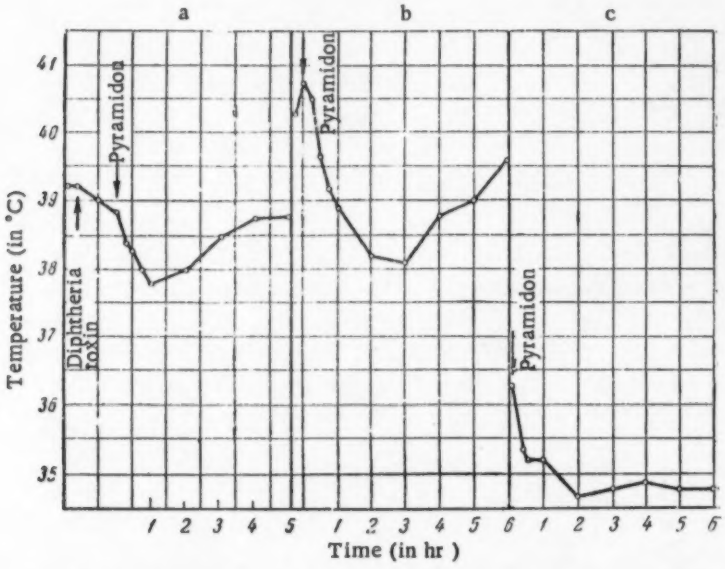

Fig. 3. Temperature change following administration of Pyramidon to rabbits which had received 3 MLD diphtheria toxin. a) 0.1 g/kg Pyramidon administered 2 hr after injection of 3 MLD toxin; b) 0.1 g/kg Pyramidon administered 24 hr after injection of 3 MLD toxin; c) 0.1 g/kg Pyramidon administered 48-72 hr after injection of 3 MLD toxin.

Pyramidon's effect was stronger at the later periods of lethal diphtheria intoxication than at the early and often caused a catastrophic fall of temperature. This further corroborates the literature data [4, 5, 6] to the effect that the functional condition of the thermoregulator apparatus is disturbed in diphtheria intoxication and indicates the great sensitivity of the injured thermoregulator center to the antipyretic.

SUMMARY

Three series of experiments were staged on rabbits. As demonstrated, Pyramidon exerts no effect on development of dinitrophenol hyperthermia; given in the same doses it reduces the temperature of healthy rabbits and especially of those with fever. The sensitivity to Pyramidon rises in toxic injury of the thermoregulation mechanisms (diphtheria intoxication). Experiments confirm the opposite nature of the physiological mechanisms underlying hyperthermia in fever and in toxic rise of thermogenesis under the effect of dinitrophenol.

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THE INITIAL PHASE OF EXCITATION IN ABSOLUTE DIFFERENTIATION AND THE PRESENCE OF FREE AMMONIA IN THE LARGE CEREBRAL HEMISPHERES OF RATS*

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The problem of internal inhibition at the present time attracts the attention not only of physiologists, but of representatives of neighboring disciplines. Continuing an investigation into the biochemistry of cortical inhibition, we observed a characteristic change in the ammonia content in the brain which testifies to the presence of definite stages in the process of development of extinctive and differential inhibition in rats [3, 4]. In particular we discovered a characteristic increase in the level of free ammonia in the brain in the first 5 seconds of activity of a negative conditioned stimulus during absolute differentiation [5, 6].

It was of interest to explain the changes in ammonia content in brain tissue in the first 5 seconds of special stable reinforced absolute differentiation, which was attained by use of a considerably larger number of repeated negative conditioned stimuli than in previous experiments.

EXPERIMENTAL METHOD

All experiments were carried out on white rats weighing 200-280 g, maintained on the same nutritive ration. In a chamber specially constructed by us [1, 2] motor-alimentary and motor-defense conditioned reflexes were developed in the rats. Electrocutaneous stimulation served as an unconditioned stimulus in the latter case.

In our development of the motor-alimentary conditioned reflexes, the isolated action of a positive conditioned stimulation (bell) consisted of 3-5 seconds, accompanied by a reinforcement of 10 seconds. The animal usually ate the portion of food given out in 45-60 seconds. The pause before contact of the bell, as in the previous experiments, was equal to 1 min, and before application of the differential stimulation (buzzer) was 2 min. The buzzer was inserted in the 3rd to the 6th place in the order of stimuli in the experiment. During development of differentiation the buzzer at first was turned on at 30 seconds, and later at 60 seconds.

The brain tissue was fixed at the requisite moment by instantaneous submersion of the animal in liquid air, using the same chamber with automatically falling bottom [1, 2]. This chamber was recently found useful in the work of Japanese authors [8] for investigation of the ammonium content of rat brain at different intervals when stimulated by electric current and by positive conditioned-reflex motor-defense reaction.

Another method of freezing the rat by immersion in a mixture of acetone and dry ice at −80° is described in the literature [7]. However, this method of freezing excludes the photometric determination of ammonia, since even traces of acetone form turbidity on Nesslerization.

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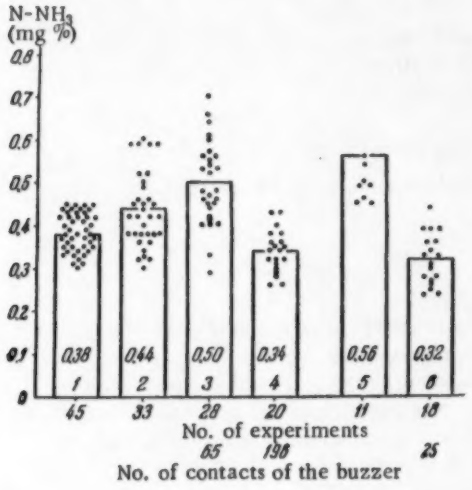
We determined the content of free amonia in the large hemispheres of brain, as in our previous experiments, by vacuum distillation with subsequent photometric determination, the color being developed with the addition of Nessler's reagent.

EXPERIMENTAL RESULTS

Differentiation in rats was sufficiently stable so that the animal in response to differential stimulation did not react either by a positive motor-alimentary reaction or by a noticeable orientation reaction after 65 contacts of the buzzer, on the average.

As was shown in previous investigations, the ammonia content of the brain after the first 5 seconds of positive conditioned-reflex motor-alimentary reaction consists, on the average, of 0.44% (see figure, 2) and after the first 5 seconds from the beginning of negative conditioned stimulus, of 0.50 mg% (see figure, 3).



Change in ammonia content in rat brain in the first 5 seconds of positive motor reaction and in the first 5 seconds of absolute differentiation during different degrees of reinforcement. The numbers on the columns indicate the average ammonia content (in milligram-percent). 1) Normal; 2) positive motor-alimentary reaction; 3) differentiation, 65 contacts of the buzzer; 4) differentiation, 196 contacts of the buzzer; 5) positive motor-defense reaction; 6) differentiation, 25 contacts of the buzzer (motor-defense reaction).

In the first series of experiments with special reinforcement of differentiation the number of contacts of positive conditioned stimulus averaged 472, and differentiation 176; the ammonia content in the large hemispheres of the brain after the first 5 seconds from the beginning of differential stimulation varied within normal limits and was 0.36 mg%, on the average.

In the second series of experiments the number of repetitions of the bell was increased to 528 and the buzzer to 207 times on the average. The ammonia content in the brain was somewhat less than in the previous series of experiments, being 0.33 mg% on the average. The average ammonia content of rat brain in the 2 following series of experiments with 509 contacts of the bell and 196 contacts of the buzzer consisted of 0.34 mg% (see figure, 4), that is, 32% less than the ammonia we found by 5 seconds from the beginning of buzzer application, but with the usual reinforcement of differentiation (see figure, 3). Decrease in the level of ammonia was statistically verified both with respect to normal level ($P < 0.01$) and even more to the amount of ammonia during positive conditioned stimulation ($P < 0.01$) (see figure, 2).

It must be noted that the appearance of absolute differentiation, reinforced either with 196 or 65 repetitions of the buzzer, was completely identical; the animals did not manifest even an orientation reaction when differential stimulation was incorporated. However, associated change in the level of free ammonia in the brain indicates a difference in the functional condition of the central nervous system in both cases.

It seems to us that the absence of specific increase in the level of ammonia in the brain in the first 5 seconds of special stable reinforced differentiation was a result of the appreciable shortening of the excitation phase and the increased concentration of inhibitory processes.

It must be noted that the ammonia content in the brain was still lower in the first 5 seconds of differentiation produced by the motor-defense method, after the use of a comparatively insignificant number of buzzer contacts (25 on the average). The ammonia in the brain equalled 0.32 mg% on the average (see figure, 6), which is 16% lower than normal (see figure, 1). This decrease in ammonia was statistically verified ($P < 0.01$) with respect to the normal and even more with respect to the amount of ammonia found in the first 5 seconds of positive conditioned reflex motor-defense reaction (0.56 mg%) (see figure, 5). An increase in the level of

ammonia to an average of 0.54 mg% occurred in only 7 cases out of 25. These were animals with insufficiently established differentiation (18 buzzer contacts with nonreinforced electrocutaneous excitation).

When the order of distribution of ammonia values presented in the figure is examined, it is not difficult to see that during the first 5 seconds of the usual stable differentiation by means of the motor-alimentary method, the ammonia in the majority of cases was higher than normal, while with special stable reinforced differentiation it often lies within normal limits of ammonia or lower, as in the first 5 seconds of differentiation developed by means of the motor-defense method.

At the same time the ammonia levels in the brain in the first 5 seconds of positive conditioned-reflex motor-defense reaction were significantly higher (on the average of 0.56 mg%; see figure, 5) than with the positive motor-alimentary reaction (0.44 mg% on the average; see figure, 2).

The varying picture of change in the ammonia level in the brain took place in the first seconds of differential inhibition developed, on the one hand, by means of the motor-defense, on the other hand, by means of the motor-alimentary method. In the first case the level of ammonia decreased (0.32 mg %) and in the second became higher (0.50 mg %). And only when differentiation was specially reinforced was the same decrease in the ammonia level (0.34 mg %) found as in the first case.

On what depended the absence or non-appearance of the beginning stages of excitation during differentiation developed by means of the motor-defense method, in spite of the small number of buzzer contacts with nonreinforced electrocutaneous stimulation?

We consider it probable that extinction occurred more rapidly on electrocutaneous (painful) stimulation of the initial intermittent reaction related to the positive conditioned-reflex reaction than when differentiation is developed with the motor-alimentary method. In this case the absence of increase in the ammonia level in the brain in the first 5 seconds of differentiation developed with the motor-defense method can also be attributed to appreciable shortening of the initial stages of excitation. In addition to this, apparently, the nature of the unconditioned stimulation employed has a significant effect on ammonia metabolism in the brain and on the character of the development of differential inhibition.

The latter circumstance, probably, can explain the well-known difficulties arising from attempts to create a condition of neurosis in animals by the use of "knockdown" stimulatory and inhibitory processes by means of the motor-defense method.

Our data have a direct relationship to the problem of the mechanism of the emergence of inhibition in the brain cortex, since some moments of development of threshold, extinction, and differential inhibition are reflected in changes in the free ammonia content of the brain.

On the basis of results of the present investigation it was possible to reach the following conclusion.

Biochemical investigation of the brain facilitates discovery of differences in the metabolism of the central nervous system during the first seconds of absolute differentiation with different degrees of reinforcement employing common well-defined differential reactions. The absence of specific increase in the ammonia level in rat brain in the first 5 seconds of special reinforced absolute differentiation is dependent, apparently, on appreciable shortening of the initial phase of excitation and increased concentration of the inhibitory processes.

The degree of reinforcement of differentiation, and also the nature of the unconditioned stimulus used, have a significant effect on the process of formation of differential inhibition and ammonia metabolism in the brain.

SUMMARY

The use of Pavlov's conditioned reflex method combined with rapid fixation of intravital changes occurring in the free ammonia level of the large cerebral hemispheres of rats enabled us to discover variations in the brain metabolism already within the first 5 seconds of the absolute differentiation with various degrees of stability, the differentiation reactions being visually absolutely uniform in character. The absence of specific rise in the ammonia level in the brain during the first seconds of a particularly stable differentiation is evidently caused by a considerable shortening of the initial phase of excitation and the intensification of the inhibitory processes concentration.

It is not only the degree of stabilization of the differentiation but also the origin of the unconditioned stimulus used that exercise a considerable effect upon the process of differential inhibition and ammonia metabolism in the brain.

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THE EFFECT OF GLUTATHIONE, VITAMINS B_{12} AND B_1, AND RUTIN ON THE LEVEL OF CHOLESTEROL IN THE BRAIN, LIVER AND BLOOD IN ALIMENTARY HYPERCHOLESTERINEMIA

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In the light of existing data on the effect of lipotropic substances on cholesterol content in the blood in atherosclerosis [1, 2, 3], as well as data on glutathione inhibition of lipid accumulation in the liver in experimental hypercholesterinemia [4], we undertook to study the effect of vitamins B_1 and B_{12}, rutin, and glutathione as well on the cholesterol content in organs of chicks with experimental hypercholesterinemia.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 266 12-day-old chicks. In each series they were subdivided into 8 groups. The first group were controls; their diet consisted of wheat porridge supplemented with 2.5% sunflower oil and green grass. The chicks of the remaining 7 groups received wheat porridge supplemented with sunflower oil containing 2% cholesterol and green grass for 18 days. The amount of food eaten and the food remaining were registered every day. At the time of the experiment each chick had received at least approximately 4.7 g cholesterol.

After 18 days, feeding with cholesterol was stopped and the degree of hypercholesterinemia was determined in part of the experimental chicks.

Subsequently, the second group received the usual ration. Two γ of vitamin B_{12} (first series) and 0.1 γ vitamin B_{12} (second series) were injected subcutaneously daily in chicks of group 3. Rutin was added to the diet of group 4 chicks at a level of 100 mg/kg weight daily. In addition to rutin, group 5 chicks were subcutaneously injected with glutathione at a level of 12.5 mg/100 g weight. Group 6 chicks were injected subcutaneously with 0.2 mg vitamin B_1, group 7 with a solution of glutathione (12.5 mg/100 g weight) and group 8 with vitamin B_1 and glutathione in doses indicated above. Chicks in the first series received the preparations described above for 15 days, part of the chicks in the second series for 10 days, and the remainder for 20 days after cessation of cholesterol feeding.

At the end of the injection period the chicks were decapitated, the liver and brain removed, these organs were weighed and dried in a thermostat at 105° to a constant weight. The organs were ground into powder, extracted with ether in a Soxhlet apparatus for 2 days. Cholesterol was determined in the ether extracts of liver and brain with a colorimetric method based on the Liebermann-Burchardt reaction. The cholesterol content in the blood of the chicks was simultaneously determined.

Content of Cholesterol in Blood, Liver and Brain of Normal Chicks and Chicks Fed Cholesterol and Injected with Glutathione and Vitamins B_{12}, B_1 and Rutin under Conditions of Hypercholesterinemia (average data)

Group	Experimental conditions	Number of chicks				Concentration of cholesterol											
			after cessation of cholesterol feeding at			in blood (in mg %)				in liver (in % of dry weight)				in brain (in % of dry weight)			
		after 18 days of cholesterol feeding				after 18 days of cholesterol feeding	after cessation of cholesterol feeding at			after 18 days of cholesterol feeding	after cessation of cholesterol feeding at			after 18 days of cholesterol feeding	after cessation of cholesterol feeding at		
			10 days	15 days	20 days		10 days	15 days	20 days		10 days	15 days	20 days		10 days	15 days	20 days
First	Control				34				206				1.7				7.5
Second	After feeding cholesterol + ration	15	6	19	11	838	405	277	228	12.8	5.5	5.0	2.1	7.5	5.9	8.1	6.7
	Injection under conditions of hypercholesterinemia:																
Third	Vitamin B_{12}		5	20	12		205	322	201		2.9	6.4	1.9		6.9	7.4	7.7
Fourth	Rutin		6	13	10		403	216	202		4.7	2.7	2.4		6.7	7.9	7.3
Fifth	Rutin + glutathione		6	13	10		295	268	208		3.6	3.8	1.7		7.0	7.9	7.2
Sixth	Vitamin B_1		6	13	10		327	319	215		5.3	6.4	2.2		6.3	7.6	7.1
Seventh	Glutathione		5	13	10		356	328	224		5.4	4.6	1.6		6.4	8.0	6.7
Eighth	Vitamin B_1 + glutathione		6	13	10		324	265	216		5.4	6.4	1.7		6.9	8.6	6.9

EXPERIMENTAL RESULTS

The results obtained are presented in the table. From these data it is evident that the cholesterol content increased (from 206 to 838 mg %) 4-fold in the blood of chicks receiving it in their diet for 18 days. A considerable accumulation of cholesterol (12.8%) also occurred in the liver (control 1.7%). The amount of cholesterol in the brain did not change.

At 10 days after the cessation of cholesterol injections its level in the blood decreased 2-fold and at 20 days approached the initial value (second group). Ten days after the cessation of cholesterol feeding its content in the liver decreased 2.3 times, in the following 5 days its level did not change, and at 20 days cholesterol still was at a level of 23.5% and surpassed the content in control chicks.

After 10-day injections of 0.1 γ vitamin B_{12} under conditions of hypercholesterinemia (third group) the cholesterol concentration in the blood fell to the level of the control group. Cholesterol content in the liver was significantly decreased (2.9% in chicks receiving vitamin B_{12} in comparison with 5.5% in chicks not receiving vitamin).

In the group of chicks receiving rutin and glutathione simultaneously (fifth group) for 10 days, the cholesterol content in the blood was lower by 27% and in liver by 34.6% in comparison with the group of chicks not thus injected.

The level of cholesterol in the blood and liver of chicks receiving rutin for 15 days in the food (fourth group) was lower than in the second group (in blood by 22.1%, in liver by 46%).

No significant change in cholesterol content of blood and liver in hypercholesterinemia chicks was observed when vitamin B_1 and glutathione or both combined were injected.

None of the compounds used casued any significant variation in the cholesterol content of brain.

From these data the conclusion can be reached that injection of vitamin B_{12} and rutin plus glutathione into chicks with experimental alimentary hypercholesterinemia significantly accelerated the decrease in blood and liver cholesterol after cessation of cholesterol feeding.

SUMMARY

The paper deals with materials on the effect of glutathione, vitamins B_{12} and B_1, rutin and a combination of glutathione with vitamin B_1 or rutin on the cholesterol level in the blood, liver and brain during alimentary hypercholesterinemia. Experiments were staged on 266 chicks.

Hypercholesterinemia was provoked by cholesterol-containing diet given for a period of 18 days. After the cessation of cholesterol administration, the chicks were given vitamin B_{12} in a dose of 0.1 γ and 2 γ, 0.2 mg of vitamin B_1 subcutaneously, rutin with food – 100 mg per kg of body weight, glutathione – 12.5 mg per 100 g of weight and combinations of glutathione with rutin or with vitamin B_1 in the aforesaid doses for a period of 10, 15, and 20 days. The chicks were decapitated after suspension of this treatment. Cholesterol concentration was determined in the blood, liver and brain. As established, cholesterol feeding markedly increased its content in the blood and liver. After discontinuance of cholesterol administration its content in the blood and liver drops almost to the initial level in 20 days. The hypocholesterizing effect of vitamin B_{12} given in a dose of 0.1 γ, of glutathione-rutin combination and of rutin alone was established. There were no changes in the cholesterol content of the brain tissue.

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CONTENT OF COPPER IN BRAIN TISSUE UNDER NORMAL CONDITIONS AND IN SOME DISEASES OF THE NERVOUS SYSTEM

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The brain is one of the richest copper-containing organs of the human. Highest values have been observed in the cerebral cortex, subcortical nodes and the optic thalamus [2, 13]. In the left hemisphere the content of copper is higher than in the right [1, 5]. About 90% of the copper found in the brain is joined to proteins [1, 16]. Injection of sedative substances into the organism increases the concentration of copper in the brain [8], while excitation of the nervous system and convulsive states decreases copper concentration [1]. However at the present time almost nothing is known about the relative significance of copper in nervous system activity.

Our objective was to determine the copper content in functionally different parts of the human nervous system and changes in its concentration in the brain in some diseases.

EXPERIMENTAL METHOD

Copper was determined by emission spectra analysis. The spectrograms were analyzed on a spectroprojector and MF-2 microphotometer. A calibration curve was constructed on the basis of a salt mixture similar in composition to the brain ash [1, 2]. As an internal standard, the spectral line of cobalt was used, artifically added to the combustion mixture. The tissue ash was burned in an activated arc of an alternating current. Reproducibility of the investigation was ±8%.

EXPERIMENTAL RESULTS

Initially the copper content in brain tissues of essentially healthy individuals, accidentally killed, was determined. The reason for death in 5 cases was asphyxiation; in 2, electric trauma; in 3, destruction of organs important for life by mechanical trauma. All the dead were aged 18-32 years. Parts of the brain were taken for investigation only in thoses cases when no signs of brain disease could be found. Results of the determinations are presented in Table 1.

The highest amount of copper in the brain calculated on a fresh weight basis was found in the globus pallidus and caudal nucleus, followed in decreasing order by brain cortex, optic thalamus, and hypothalamic formations. The least copper was in the white matter of the brain hemisphere. When calculated on a dry weight basis the same relationships were maintained. Variation in copper concentration in the lobes, parietal and occipital parts of the brain cortex did not exceed 80%. A somewhat higher copper content (10-12%) was noted in

TABLE 1

Copper Content of Investigated Tissues

Investigated material	In tissues of healthy, accidentally killed individuals		In tissues of a patient with hepatolenticular degeneration		In tissues of patients with brain tumor	
	in milligrams/ /100 g fresh matter	in milligrams/ /100 g dry matter	in milligrams/ /100 g fresh matter	in milligrams/ /100 g dry matter	in milligrams/100 g fresh matter	in milligrams/100 g dry matter
Brain cortex	0.6 (0.43-0.92)	3.33 (2.3-3.7)	1.64	10.4	0.76 (0.41-0.9)	3.62 (3.2-4.4)
White matter	0.5 (0.4-0.7)	1.52 (1.2-2.0)	1.32	4.49	0.46 (0.26-0.8)	1.66 (1.5-1.8)
Optic thalamus	0.56 (0.51-0.71)	2.49	1.45	7.01	–	–
Caudal body	0.64 (0.49-0.75)	3.37 (3.1-3.5)	1.38	6.42	–	–
Hypothalamus	0.53 (0.31-0.71)	2.71 (2.1-3.5)	–	–	–	–
Globus pallidus	1.31 (0.73-1.68)	4.31 (3.9-4.6)	2.89	18.71	–	–
Cerebellum	–	–	1.43	8.25	–	–
Spinal cord (cervical swelling)	–	–	0.98	3.99	–	–
Spleen	–	–	0.58	2.96	–	–
Muscle	–	–	0.25	1.07	–	–
Liver – Acutely infected lobe	–	–	3.20	13.69	–	–
Liver – Less infected lobe	–	–	1.66	5.92	–	–

Note: Limits of individual variation are placed in parentheses.

the anterior hypothalamic region (sections near the intersection of the optic nerves and the anterior portion of the tuber cinereum) in comparison with the posterior (region of the mammillar bodies and the posterior section of the tuber cinereum). The lateral portions of the optic thalamus contained 0.48 mg % of copper and the medial portions 0.64 mg %.

We then investigated the copper content in tissues of a patient dying from hepatolenticular degeneration. The patient had the tremulus-rigid form of hepatolenticular degeneration. Kayser-Fleischer's ring was present, as well as disturbance of the copper metabolism. On autopsy, changes in liver and brain characteristic of hepatolenticular degeneration were found.

The copper content in the brain of the patient with hepatolenticular degeneration (see Table 1) when compared to the level of copper in the corresponding brain sections of healthy individuals accidently killed

TABLE 2

Content of Copper in Brain Tumors (in milligram-percent/100 g tissue)

Investigated tumor	Form of investigated material	In the whole tumor (average of the copper concentration in the center and on the periphery of the tumor)	In the center of the tumor	On the periphery of the tumor
Arachnoid-endothelioma	Fresh	0,221 (0,145-0,357)	0,237 (0,137-0,555)	0,206 (0,134-0,39)
	Dry	1,145 (0,91-1,57)	1,049 (0,83-1,42)	1,24 (0,85-1,88)
Benign neuroectodermal tumor	Fresh	0,44 (0,138-1,05)	0,366 (0,152-0,985)	0,507 (0,23-1,12)
	Dry	1,418 (1,045-1,755)	1,209 (1,070-1,31)	1,627 (0,84-2,20)
Malignant neuroectodermal tumor	Fresh	0,823 (0,575-1,011)	0,45 (0,184-0,802)	1,19 (0,77-1,58)
	Dry	4,955 (4,7-5,15)	2,53 (1,0-4,060)	7,38 (6,24-8,52)

when calculated on a fresh weight basis, was 274% in the cortex , 264% in the white matter, 259% in the optic thalamus, 220% in the globus pallidus and 216% in the nucleus caudatus. A similar comparison of the copper concentration in the relative parts of the brain calculated on a dry weight basis showed a somewhat different relationship: 191% in the nucleus caudatus, 281% and 292% respectively in optic thalamus and white matter, 312% in brain cortex and 434% in globus pallidus.

Due to the fact that a patient with hepatolenticular degeneration is rarely autopsied, copper determination in the cerebellum,spinal cord, liver and muscles of this patient are also presented in Table 1.

Finally, in the last series of investigations we determined the copper content in neoplasms and tissues of the nervous system in brain tumors.*

Twenty-one tumors were investigated, of which 11 were arachnoidendothelial, 8 were neuroectodermal tumors of various forms (3 spongioblastoma multiforme and 5 astrocytomas), one was a tumor of hypophysis (chromophilic adenoma), one was an epidermoid cyst. Sections of the brain not infected with tumor were investigated in 9 cases. Tissues free of vessels and decomposed matter were used in the determination.

A small amount of copper (Table 2) was found in tumors of the membrane-vascular system (arachnoidendothelial). In intracerebral benign tumors (astrocytomas) the amount of copper approached that in white matter of the brain. The most copper was contained in the malignant neuroectodermal tumors (spongioblastoma multiforme). This difference was maintained when the copper content was calculated on a dry weight basis. In the case we investigated of intracerebral tumors with a noncerebral origin (epidermoid cyst), the copper content exceeded somewhat the concentration in arachnoidendothelial tumors (0,246 mg %). A very small quantity of copper was found in the chromophobic adenoma of the hypophysis (0,104 mg % calculated on fresh tissue and 0,320 mg % by dry weight).

* Parts of the brain were obtained at the time of operation, when access to the tumor was accompanied by the necessary removal of uninfected tissue, and in some cases brain sections were obtained at autopsy. The specimens were obtained from the Neurosurgical Department (Director, Dr. Med. Sci. I. M. Irger) of the Scientific Research Institute of Psychiatry of the Acad. Med. Sci. USSR, in the S. P. Botkin Base Hospital.

Different groups of tumors also differed with respect to distribution of copper in various parts of the neoplasms. In arachnoidendotheliomas the variation in copper content in various parts did not exceed 10-15% as a rule. In benign neuroectodermal tumors the copper content on the periphery of the tumor was 29% higher on the average than in the center. In malignant penetrating tumors of the brain this difference attained 62% by wet weight, and 290% calculated by dry weight.

The copper content (see Table 1) in the white matter not infected with tumor (in milligram-percent fresh weight) was lower and in the cortex (in 5 observations out of 6) it was higher than the average copper concentration in the brain of healthy individuals. The copper content (dry weight) of both cortex and white matter exceeded the average copper content in healthy, suddenly-killed individuals.

The values presented above for copper in the brain of suddenly-killed, essentially healthy people verifies data obtained by other investigators [2, 13] regarding the unequal copper content in various parts of the brain. The high content of copper in the brain of the patient dying from hepatolenticular degeneration verifies existing observations of the disturbance of copper metabolism in this disease [7, 12, 13].

The unequal accumulation of copper in various parts of the brain should be noted. The highest concentration of copper in normals and in hepatolenticular degeneration was noted in the globus pallidus and the nucleus caudatus.

In order to determine the significance of copper in tissues of the nervous system it is appropriate to compare the data presented above regarding the high copper concentration in the subcortical nodes of the brain with the facts indicating the ability of the copper-containing proteins of the blood serum to acidify some catecholamines (adrenalin, noradrenalin and others [4, 15]) and observations of the relationship of the serum copper content to changes in the tonus of the sympathetic nervous system [3, 4]. It is also known that in disease of the ganglia at the base of the brain secretion of catecholamines from the urine are appreciably increased. Isolated destruction of the globus pallidus in such patients leads to still higher increase in the synthesis of these amines in the urine. Analogous activity in other parts of the brain does not have such an affect [11]. Injection of drugs which act on the metabolism of catecholamines in the brain (aminazine and ganglioblocking agents) leads to the development of a syndrome of injury to the subcortical ganglia of the brain in those patients who have a high copper blood level at the time of treatment [10]. As our investigation showed, patients with organic disease of the subcortical ganglia of the brain have an increased copper blood level.

It is difficult to imagine that this chain of events is the result of accidental coincidence. It is more probable that there is a relationship between the copper-containing proteins in the brain and the catechol amines, adrenalin and noradrenalin in particular, in subcortical ganglia. From this point of view, interest is drawn to the fact noted above that the copper content is significantly higher in medial sections of the optic thalamus than in the lateral sections. It was shown [17] that the content of sympathins (adrenalin and noradrenalin) in medial sections of the optic thalamus is also higher than in lateral sections.

An almost equivalent amount of copper (only 2-4% higher than experimental error) in the anterior and posterior sections of the hypothalamus coincides with a uniform distribution of sympathins in these formations [17]. Therefore, definite correlation between copper content and catecholamines occurs in other parts of the nervous system.

Some increase in copper, which we noted in parts of the cerebral matter not infested with neoplasm in patients with brain tumors, was entirely insignificant and occurred in small samples.

The copper content of brain tumors with varying histogenesis varied within wide limits. The smallest amount was noted in tumors of the membrane-vascular series and benign neuroectodermal neoplasms. In spongioblastoma multiforme the copper concentration exceeded that of the cortex and on a dry weight basis it was higher than the maximum copper content in globus pallidus. If the concentration of copper in the center of the tumor did not exceed the value for white matter of the brain, then the peripheral part of the tumor contained approximately 200% more copper than the surrounding tissue. In malignant neuroectodermal tumors the amount of copper in the periphery was 2.9 times higher than in the center.

These data cast doubt on the opinion expressed by some authors [6] that increase in the copper concentration in tumors is related to the development of foci of necrosis in them. As is known, the intensity of cellular growth, anaerobic glycolysis and the content of nucleic acids is higher in malignant tumors, and also larger in the

periphery of neoplasms [9]. Comparison of the results obtained with data in the literature lead us to the conclusion that the parts of the tumors with increased copper content are at the same time tissues with the most intensive metabolism and cellular growth, which is evidence of the importance of copper and copper containing proteins for metabolic processes proceeding in tumors.

SUMMARY

Copper content was determined spectrographically in various portions of the brain in 10 healthy patients who died accidentally, in the brain of a patient with hepatolenticular degeneration, as well as in various tumors of the brain and areas of the nervous system not affected by neoplasms. The greatest copper content was noted in the globus pallidus, nucleus caudatus and the cortex of the large hemispheres. The copper concentration in the brain of patients with hepatolenticular degeneration is considerably higher than in the accidentally dead persons. As shown, less copper is contained in the lateral portions of the optic thalamus than in the medial portions. The concentration of copper was almost even in the anterior and the posterior portions of the hypothalamic area. Copper content is increased in the areas of the brain not affected by neoplasms. Copper content in the brain tumors of membranous-vascular genesis is lower than in the white matter. Much copper was detected in benign neuroectodermic tumors. The highest copper content was noted in malignant neuroectodermal tumors (higher than in the brain tissue richest in copper). In malignant tumors copper content was higher at the periphery of the neoplasm, in the area of intensive cellular growth and glycolysis. The significance of copper for the actvity of the nervous system and metabolism in tumors is discussed.

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THE EFFECT OF PENTOXYL ON THE BONE MARROW LIPIDS OF RABBITS WITH EXPERIMENTAL LEUCOPENIA

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The object of the present investigation was to study the changes in content of total lipids, phospholipids and cholesterol in bone marrow of rabbits with experimental leucopenia induced by Embichin, as affected by an increase in myeloblastic function of the bone marrow by pentoxyl – a stimulator of myelopoiesis [1].

EXPERIMENTAL METHOD

The experiments were carried out on rabbits 3-4 months old.

In the first series of experiments the injection of Embichin inhibited myelopoiesis in animals [2]. Embichin was injected intravenously daily in physiological solution at a dose of 0.3 mg/kg body weight. With the onset of distinct leucopenia accompanied by granulocytopenia, the injection of Embichin was stopped and pentoxyl was injected at a daily subcutaneous dose of 15 mg/kg body weight. When total leucocytes and granulocytes in peripheral blood attained a normal value, the animals were sacrificed. Twenty experiments in all were carried out with pentoxyl. Rabbits killed when acute leucopenia and granulocytopenia developed served as controls.

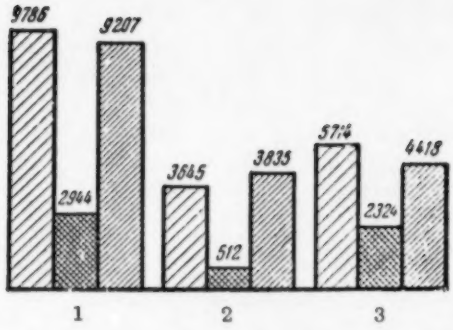
The bone marrow of normal rabbits (28 animals) was studied simultaneously.

The total bone marrow of the epiphysis and diaphysis of the femoral bones was removed for the investigation.

Extraction and determination of the lipids was carried out in the following manner. A weighed portion of ground bone marrow (from 2 to 3 g) was extracted at room temperature with ethyl alcohol, followed by ether, each for 1 hour; finally the marrow was extracted with a boiling mixture of chloroform and methanol (1:1) for 4 hours and returned to the refrigerator. The solvent was boiled off from the combined alcohol-ether-methanol-chloroform extract on a water bath at reduced pressure and in a current of CO_2. The lipids were extracted from the half-dry residue obtained with petroleum ether. The solvent was boiled off from the petroleum ether extract in a current of CO_2 with reduced pressure. The residue obtained was dried in a vacuum desiccator over $CaCl_2$, after which the total lipids were determined by weighing. The total lipids were dissolved in a mixture of alcohol, ether, methanol and chloroform and the phospholipid and cholesterol content determined in specific parts of this extract. The phospholipids were precipitated and fractionated into a readily hydrolyzable fraction and a fraction hydrolyzable with difficulty according to the method of Hack [7]. The phosphorus of the total and readily-hydrolyzable phospholipids was determined after combustion with H_2SO_4 and $HClO_4$ according to the Fiske-Subbarow method. The phosphorus of the phospholipids hydrolyzable with difficulty was calculated by the difference between the content of phosphorus in the total and easily-hydrolyzable phospholipids. Cholesterol was determined according to the Schoenheimer and Sperry method.

EXPERIMENTAL RESULTS

Leucopenia developed in all rabbits (see figure) with injection of Embichin. Significant change in the lipid content of bone marrow was also noted.



Content of leucocytes, pseudoeosinophils and lymphocytes in blood of intact rabbits (average of 16 experiments), rabbits receiving Embichin (average 12 experiments) and rabbits receiving pentoxyl (average of 19 experiments). Light columns, intact rabbits; cross-hatched columns, rabbits receiving Embichin; densely striped columns, rabbits receiving pentoxyl. 1) Total number of leucocytes; 2) absolute content of pseudoeosinophils; 3) absolute content of lymphocytes.

As seen from the data presented in Table 1, total lipids decreased on the average to one-half the normal level, and furthermore in certain cases the level was extremely low (experiment No. 2). This decrease always accompanied an increase in water content. The dry residue in the bone marrow free from lipids changed only slightly, and decreased only in those cases where the number of leucocytes decreased to 1000 and less in 1 ml blood (experiment No. 4).

When pentoxyl was injected (Table 2) an increase in the bone marrow myelopoiesis was observed in all cases, with the exception of one (experiment 20), after only 3-4 days (see figure). The total leucocytes in peripheral blood increased 3.1 times on the average in comparison with leucocytes in Embichin leucopenia; at the same time the absolute number of granulocytes (pseudoeosinophils) increased 7.5 times, attaining a normal value.

A predominance of pseudoeosinophils over lymphocytes was observed in a number of cases. The condition of the animals during the whole period remained good.

The content of bone marrow lipids changed at the same time. The results obtained were very similar, so that in Table 2, data from only some of the experiments are presented.

As seen in Table 2, total lipids in the bone marrow of rabbits receiving pentoxyl varied from 23.9 to 48% and averaged 35.8%. The dry residue free from lipids varied within narrow limits, equalling 13.8% on the average. The phosphorus in the phospholipids averaged 32.7 mg %, while the phosphorous of phospholipids hydrolyzed with difficulty varied from 3.5 to 15 mg %, averaging 11 mg %.

Comparing the chemical compounds of the bone marrow of rabbits receiving Embichin and pentoxyl, it can be noted that under the influence of pentoxyl increase in the number of granulocytes (pseudoeosinophils) in the peripheral blood was accompanied by a 1.5-fold increase in total lipids, which almost reached the normal value. Such a significant increase in the amount of total lipids can originate only at the expense of neutral fat, since neutral fat comprises a large part of the bone marrow lipids.

When the development of leucopenia progressed (experiment No. 20) in spite of pentoxyl injection and the number of leucocytes in the peripheral blood decreased to 850,000 in 1 mm^3, the lipid content in bone marrow decreased. This change in fat content in bone marrow and concomitant disturbances of its erythroblastic function has been noted by a number of authors [3, 6, 8, 9].

These data indicate that the content of neutral fat in bone marrow is dependent on the condition of its hematopoietic function. It is possible that active metabolism of fat occurs in the bone marrow, since it has been established that synthesis of fatty acids from labeled acetate occurs in bone marrow homogenates [4, 5].

The phospholipid content of bone marrow varies with respect also to the state of myelopoiesis. In rabbits receiving pentoxyl, the phosphorus content of phospholipids in bone marrow increased 1.5-fold in comparison with the phosphorus content of this fraction during Embichin leucopenia. Furthermore, its value is higher than normal. It is known that phospholipds enter into the composition of formed elements. Consequently, it is possible that during restoration of myelopoiesis in the bone marrow, an accumulation of phospholipids takes place – phospholipids being a substrate necessary for the formation of formed elements.

The water content in the bone marrow of rabbits receiving pentoxyl varied widely. In normal animals, in Embichin-leucopenia animals and in animals injected with pentoxyl the water content was found to be in reverse proportion to the lipid content.

TABLE 1

Composition of Bone Marrow of Femoral Bones of Rabbits with Embichin Leucopenia, Not Receiving Pentoxyl (calculated on wet weight of tissue)

Expt. No.	Time of determination	Hemoglobin (in %)	Erythrocytes in blood (10^6)	Leucocytes in blood (10^3)	Wt. of animal (in g)	Weight of bone marrow (in g)	Water (in %)	Lipids (in %)	Dry residue, free from lipids (in %)	Phosphorus of phospholipids (in mg %)			Cholesterol, total (in mg %)
										total	easily hydrolyzed	hydrolyzed with difficulty	
1	Before expt.	68	4 540	7 300	1 720	3.24	68.3	19.7	12.0	22.7	16.2	6.5	113.6
	6 days after Embichin injection	58	3 720	2 900	1 650								
2	Before expt.	70	6 430	10 900	2 150	3.43	84.8	5.0	10.2	23.9	17.6	6.3	121.3
	6 days after Embichin injection	75	5 870	3 100	1 950								
3	Before expt.	70	5 460	9 720	1 850	2.71	55.1	33.7	11.2	28.6	22.8	5.8	97.0
	7 days after Embichin injection	72	5 290	2 700	1 670								
4	Before expt.	72	4 910	8 550	1 870	3.05	52.4	39.3	8.3	13.4	9.3	4.1	84.9
	7 days after Embichin injection	65	4 550	550	1 870								
Av. of 15 expt.	Before expt.	67	5 006	9 789	1 861	3,10 (2.34—4.07)	64.9 (51.3—84.8)	23,7 (5.0—39.3)	11.3 (8.1—14.6)	22.1 (13.4—29.9)	16.0 (9.3—24.4)	6.1 (2.1—8.3)	111.5 (84.9—133.2)
	After Embichin injection	67	4 640	2 944	1 748								
Control rabbits (average values of data obtained from 28 animals)		70	5 142	9 174	1 900	3.70 (2.10—5.68)	46.0 (38.9—68.8)	42.0 (17.0—50.7)	12.1 (9.2—14.2)	27.3 (20.6—33.2)	21.4 (15.5—26.5)	5.9 (2.22—8.68)	101.6 (45.0—137.8)

TABLE 2

Composition of Bone Marrow of Femoral Bones of Rabbits with Embichin Leucopenia, Receiving Pentoxyl (calculated on wet weight of tissue)

Expt. No.	Time of determination	Hemoglobin (in %)	Erythrocytes in blood (10^6)	Leucocytes in blood (10^3)	Weight of animal (in g)	Wt. of bone marrow (in g)	Water (in %)	Lipids (in %)	Dry residue, free from lipids (%)	Phosphorus of phospholipids (in mg%)			Cholesterol (in mg%)
										total	easily hydrolyzable	hydrolyzable with difficulty	
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Before the experiment	65	3 840	7 350									
	6 days after Embichin injection	65	3 740	1 425									
	3 days after pentoxyl injection	60	3 550	8 450	1 900	4.44	42.2	48.0	9.8	28.2	20.7	7.5	123.2
2	Before the experiment	55	4 240	6 750	1 650								
	6 days after Embichin injection	57	4 220	2 150	—								
	3 days after pentoxyl injection	55	4 045	6 575	—	2.49	51.0	33.5	15.5	45.9	38.7	7.2	—
3	Before the experiment	70	5 430	9 930	1 650								
	6 days after Embichin injection	58	4 560	4 725	—								
	3 days after pentoxyl injection	70	5 040	8 150	1 580	3.71	45.2	42.8	12.0	30.8	20.3	10.5	109.1
4	Before the experiment	69	5 080	7 650	1 750								
	6 days after Embichin injection	69	4 570	3 200	1 620								
	3 days after pentoxyl injection	61	4 645	8 125	—	3.35	63.6	23.9	12.5	36.45	—	—	112.8
5	Before the experiment	73	5 315	10 475	1 620								
	6 days after Embichin injection	67	4 660	2 575	1 570								
	3 days after pentoxyl injection	66	5 220	14 450	1 550	2.16	47.3	39.1	13.6	36.1	21.4	14.7	76.3
6	Before the experiment	71	5 350	7 400	1 900								
	6 days after Embichin injection	67	5 240	2 925									
	3 days after pentoxyl injection	84	5 115	10 850	1 930	2.41	52.6	31.8	15.6	37.4	33.9	3.5	62.1

(concluded on next page)

TABLE 2 (concluded)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
7	Before the expt.	67	4 870	9 625	1 920								
	6 days after Embichin injection	66	4 775	2 225	1 900								
	3 days after pentoxyl injection	69	5 210	6 900	1 950	3,28	59,1	25,4	15,5	36,0	20,9	15,1	105,2
8	Before the expt.	79	5 080	9 325	2 300								
	6 days after Embichin injection	74	4 810	1 550	—								
	3 days after pentoxyl injection	70	4 675	9 275	2 320	4,67	40,1	46,3	13,6	24,9	14,2	10,7	102,8
	Av. value of 19 expts.	70	4 910	9 207	1 878	3,07	50,4 (40,1—63,6)	35,8 (23,9—48,0)	13,8 (9,8—15,7)	32,7 (24,9—45,9)	21,7 (13,9—38,7)	11,0 (3,5—15,0)	99,5 (62.1—125.1)
20	Before the expt.	76	5 110	13 850	2 180								
	6 days after Embichin injection	65	5 100	3 250									
	3 days after pentoxyl injection	65	5 005	850	1 800		71,9	19,6	8,5	13,4	8,8	4,6	108,1

Note: The minimum and maximum values are presented in parentheses.

On the basis of data obtained, it can be concluded that when pentoxyl is injected into rabbits with Embichin leucopenia, an increase of granulocytes in the peripheral blood is accompanied by an increase in the total lipids in the bone marrow mainly at the expense of the neutral fat fraction and the phospholipids. It can be assumed that these changes are related to the myeloblastic function of bone marrow.

SUMMARY

A rise of the total lipid content was noted in the bone marrow of rabbits with Embichin leucopenia during pentoxyl administration with the rise of the pseudoeosinophil count (granulocytosis) in the peripheral blood. The rise of the lipid content occurs mainly at the expense of the increase of the neutral fat and phospholipid fraction. It may be suggested that these changes occur in connection with the bone marrow myelopoiesis.

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THE INFLUENCE OF PHENOTHIAZINE DERIVATIVES ON THE ACTION OF ANALGESICS

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The literature contains several articles on the use of phenothiazine derivatives in combination with various medicinal substances: analgesics [5], somnifacients and narcotics [1, 3] muscle relaxants and anesthetics [2, 4]. However, opinion on the interaction of neuroplegic with analgesic agents is divided. Some authors note that potentialization occurs when phenothiazine derivatives are used in combination with analgesics [5, 8], while others deny this.

In order to determine how phenothiazine derivatives intereact with analgesics, we studied various combinations of Mepazine [10-(1'-methylpiperidyl-3-methyl) phenothiazine acetate; Pacatal], Aminazine [chlorpromazine] and Propazine [10-(2'-dimethylaminopropyl) phenothiazine hydrochloride; promazine] with morphine, Promedole [4-phenyl-4-propoxy-1,2,5-trimethyl-piperidine hydrochloride; Trimeperidine] and Phenadone [methadone].

The experiments were performed on white rats by determining the absolute threshold of the animals to faradic current. Stimulation was applied by needle electrodes inserted under the skin of the tail. Since our observations have given us excellent reasons to believe that a squeak from these animals is a sign of pain, the squeak was used as the index of the reaction.

Statistical processing was carried out in order to determine the authenticity of the difference between the arithmetic means of the control (maximal effect of the individual ingredients in double doses) and the experiment (maximal effect of the pharmacological combination).*

Effect of Mepazine combined with analgesics: For combination with analgesics, Mepazine was used in a dose of 50 mg/kg; neither this dose nor double its amount produced an analgesic effect (Fig. 1). The administration of 5 mg/kg morphine caused a marked decrease in the pain sensitivity of the rats. This effect was considerably enhanced on a background of Mepazine administration. The mixture of the two substances had an effect which was not only stronger than the sum of the effects of its ingredients, but was also stronger than that of double the morphine dose.

The most effective combination was Mepazine and Promedole. Promedole was used in a dose of 2 mg/kg in this combination. The efficiency of Promedole was more than quadrupled after the preliminary administration of Mepazine; this combination also produced a stronger analgesic effect than double the Promedole dose. The absolute threshold became over 10 times higher in a few animals.

We tested two combinations of Phenadone and Mepazine. The first combination used a 1.25 mg/kg dose of Phenadone; the second, double this dose. The absolute pain threshold of the animals increased 4.5 times with the use of the first combination, but only 2.5 times with the second combination using the double Phenadone dose. The intensification of the analgesic effect obtained with the use of the second combination was less significant.

* The authenticity criterion was D = 0.05.

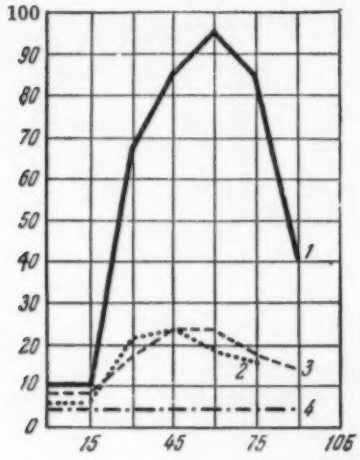

Fig. 1. Effect of Mepazine-Promedole combination on the pain sensitivity of white rats. Change in pain sensitivity after administration of: 3) 2 mg/kg Promedole; 2) 4 mg/kg Promedole; 4) 50 mg/kg Mepazine; 1) 50 mg/kg Mepazine and 2 mg/kg Promedole. Ordinate axis: absolute threshold of electric stimulation (in volts); abscissa axis: time of experiment (in minutes).

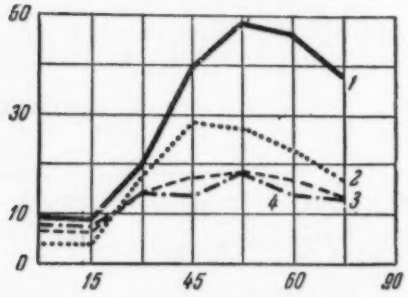

Fig. 2. Effect of simultaneous use of Aminazine and morphine on the pain sensitivity of white rats. Change in pain sensitivity after administration of: 3) 5 mg/kg morphine; 4) 5 mg/kg Aminazine; 2) 10 mg/kg morphine; 1) 5 mg/kg Aminazine and 5 mg/kg morphine. Ordinate axis: absolute threshold of electric stimulation (in volts); abscissa axis: time of experiment (in minutes).

The effect of the Mepazine-Phenadone combination was three times as strong as that of Phenadone alone, but in comparison with the efficiency of the latter preparation in a dose of 5 mg/kg, the difference was statistically unreliable.

Combined effect of Aminazine and analgesics: In a dose of 5 mg/kg, Aminazine caused a substantial decrease in the pain sensitivity, approximately equal to that effected by a like amount of morphine. A combination of these preparations was even more effective (Fig. 2). The effect of this combination was not only stronger than that of the sum of its ingredients, but also stronger than the effect of twice the dose of its most active component, morphine. The most active combination was found to be that with Promedole, as in the experiments with Mepazine. Promedole was used in a dose of 2 mg/kg in the combination. After the administration of this mixture, the pain sensitivity of the animals decreased to less than 1/6 the original. The effect obtained from a combination of the two preparations was 1.5 times stronger than the analgesic effect of twice the dose of Promedole.

A dose of 2.5 mg/kg Phenadone was used in combination with Aminazine. This combination reduced the sensitivity to painful stimulation 7.4 times. The effect of this mixture was considerably stronger both than the sum of its ingredients' effects and than the effect of double the Phenadone dose.

Combined effect of Propazine and analgesics: A 10 mg/kg dose of Propazine caused a sharp decrease in the pain sensitivity of the rats, equal to that caused by 5 mg/kg morphine (Fig. 3). After the preliminary administration of Propazine in this dose, morphine, Promedole and Phenadone (used respectively in doses of 5, 2 and 2.5 mg/kg) produced an analgesic effect considerably stronger than that of double the dose of each analgesic agent, used alone.

An especially enhanced effect was produced by the combinations with Promedole and morphine, while the combination with Phenadone was less active.

Narcotic effect of phenothiazine derivatives combined with analgesics: The use of neuroplegic agents in combination with analgesics also exhibited reciprocal enhancement of effect in relation to the sleep phenomenon. When the analgesics were administered to mice, the development of the effect was not attended in any case by loss of the "righting reflexes" or by a passive dorsal position of the animals. In these experiments, Promedole, Phenadone and morphine were used respectively in doses of 10, 20 and 40 mg/kg intraperitoneally administered.

Experimental Results Showing the Analgesic Activity of Pharmacological Combinations and Their Components

Pharmacological agents (in mg/kg)	Number of observations	Threshold voltage after administration of preparations
Morphine, 5 mg/kg	18	18 ± 2.6
Morphine, 10 mg/kg	10	27 ± 4.3
Promedole, 2 mg/kg	11	132 ± 4.1
Promedole, 4 mg/kg	8	150 ± 3.3
Phenadone, 2.5 mg/kg	9	87 ± 2.6
Phenadone, 5 mg/kg	15	29 ± 5
Aminazine, 5 mg/kg	27	75 ± 1
Aminazine, 10 mg/kg	10	18 ± 0.08
Propazine, 10 mg/kg	32	16 ± 1.8
Propazine, 20 mg/kg	10	25 ± 2.4
Aminazine, 5 mg/kg + morphine, 5 mg/kg	16	42 ± 5.6
Aminazine, 5 mg/kg + Promedole, 2 mg/kg	16	78 ± 10.8
Aminazine, 5 mg/kg + Phenadone, 2.5 mg/kg	15	48 ± 10.6
Propazine, 10 mg/kg + morphine, 5 mg/kg	10	87 ± 15.4
Propazine, 10 mg/kg + Promedole, 2 mg/kg	9	87 ± 15.7
Propazine, 10 mg/kg + Phenadone, 2.5 mg/kg	14	56 ± 6
Mepazine, 50 mg/kg + morphine, 5 mg/kg	10	41 ± 9.7
Mepazine, 50 mg/kg + Promedole, 2 mg/kg	15	90 ± 13.8
Mepazine, 50 mg/kg + Phenadone, 1.25 mg/kg	10	26 ± 3.1
Mepazine, 50 mg/kg + Phenadone, 2.5 mg/kg	10	29 ± 4.7

With the use of 40 mg/kg Aminazine or of the same dose of Propazine, none of the animals remained in the dorsal position for long. As in the other experiments, the phenothiazine derivatives were injected subcutaneously into the animals. Not more than 0.6 ml of the solutions of the preparations was administered. In these experiments, not one (out of 20) animals was observed in the dorsal position after the administration of Propazine. One out of ten mice maintained the dorsal position for 15 minutes after the administration of Aminazine.

A somnifacient effect was induced by the aministration of 20 mg/kg Aminazine in combination with 5 mg/kg Promedole, 1.25 mg/kg Phenadone or 10 mg/kg morphine. All the experimental mice remained in the dorsal position. Aminazine used with morphine induced sleep lasting an average of 126 ± 16 minutes; with Promedole, the sleep lasted an average of 112 ± 13 minutes;and with Phenadone, 106 ± 10 minutes.

When the same doses of the analgesics were combined with 40 mg/kg Propazine, the somnifacient effect induced was shorter and less constant.

Propazine in combination with Phenadone, for example, induced sleep lasting an average of 54 ± 11 minutes in nine out of ten animals. The combinations with Promedole and morphine were less active. After the administration of Propazine combined with Promedole, the dorsal position was observed in four out of ten mice, and the effect lasted an average of 95 ± 20 minutes. The combined administration of Propazine and morphine caused a somnifacient effect in only three out of ten animals, lasting an average of 106 ± 43 minutes.

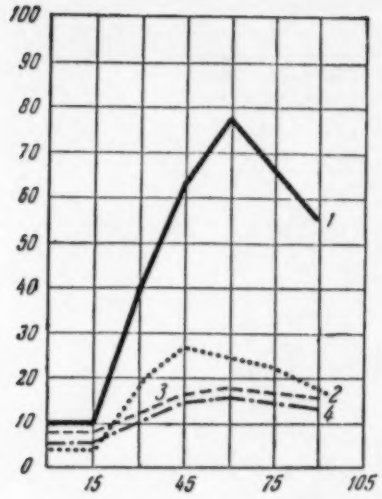

Fig. 3. Effect of Propazine-morphine combination on the pain sensitivity of white rats. Change in pain sensitivity after administration of: 3) 5 mg/kg morphine; 2) 10 mg/kg morphine; 4) 10 mg/kg Propazine; 1) 10 mg/kg Propazine and 5 mg/kg morphine. Ordinate axis: absolute threshold of electric stimulation (in volts); abscissa axis: time of experiment (in minutes).

Analogous results were observed in experiments conducted on rats, although larger doses of the analgesics were required in these experiments, and the effect was less constant. For example, the administration of 20 mg/kg Aminazine combined with 20 mg/kg morphine caused a lateral position lasting 27 ± 4.5 minutes in six out of ten rats. When the same dose of Aminazine was combined with 5 mg/kg Promedole, a somnifacient effect lasting 33 ± 5.6 minutes was observed in seven out of ten animals. Phenadone was found to be somewhat more active. The combination of 20 mg/kg Aminazine and 5 mg/kg Phenadone caused a condition of sleep lasting an average of 99 ± 4.5 minutes in all the animals.

The observations conducted have shown that an enhanced analgesic effect is obtained with the use of phenothiazine derivatives in combination with analgesics, although the effect is not equally enhanced by all the experimental combinations. For example, a combination of Mepazine and Phenadone (in a ratio of 1: 0.05) produced an only slightly enhanced effect which was no stronger than that obtained with the use of double the dose of the mixture's most active compound, Phenadone. It should be remembered, however, that Mepazine had no analgesic effect in either the dose used in the combination with the analgesic or in twice this dose.

In judging the efficiency of this mixture, therefore, the efficiency of the "most active" compound is no criterion, since only one of the preparations in this combination is active, i.e., Phenadone, and the increased activity observed can only indicate potentialization. In the other experiments, the difference between the activity of the pharmacological combinations and that of their ingredients (in double doses) was statistically significant.

The development of the narcotic effect also testified to the mutual potentialization of effect obtained with the combined use of phenothiazine derivatives and analgesics.

SUMMARY

The analgesic effect of the phenothiazine series derivatives (Mepazine, Aminazine and Propazine) and of analgesics (morphine, Promedole and Phenadone) was studied by the method of electric pain stimulation.

The presence of analgesia potentiation was established. Combinations of phenothiazine derivatives with Promedole proved most effective. Bilateral potentiation during concurrent use of phenothiazine derivatives and analgesics is also manifest in the appearance of hypnotic effect.

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THE COMPARATIVE ANTICONVULSANT ACTIVITY OF PHENOTHIAZINE DERIVATIVES IN EXPERIMENTAL ELECTRICAL SHOCK

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The question of the anticonvulsant properties of Aminazine and other phenothiazine derivatives has interested many researchers; the answers to this question, however, are still incomplete and, to some extent, contradictory. This is particularly true of works in which electrical shock has been used to reproduce a convulsive attack. For example, Hauschild [7], Tanaka and Kawasaki [12] and also Haas [6] found that chlorpromazine and certain other phenothiazine derivatives possess anticonvulsant activity. Conversely, other authors have shown chlorpromazine to be ineffective for convulsions of this type [4, 9]. The clinical and experimental data recently published on the so-called convulsant effect of chlorpromazine [2, 14] have revived interest in this question. Its practical significance is obvious due to the fact that Aminazine and other phenothiazine derivatives are often used in psychiatry to treat diseases of a convulsive nature [3].

The purpose of this work was to study Aminazine and other phenothiazine derivatives as to their ability to alter the course of a convulsive attack induced by electrical shock and to compare them in this respect with certain antiepileptics – Luminal and Diphenin [5,5-diphenylhydantoin sodium].

EXPERIMENTAL METHOD

We used the method of electrical shock* [13], modified for white mice [11] to judge the anticonvulsant action of the substances. Fifty ma alternating current was passed through the animal's head with a frequency of 50 cps and stimulation duration of 0.2 second with the aid of corneal electrodes. The convulsive attack developed rapidly; a long phase of tonic extension of the posterior extremities dominated the picture of the attack, later giving way to a phase of clonic convulsions. Absence of the tonic extension phase of the attack served as a criterion of the anticonvulsant effect. The central relaxation of the skeletal musculature characterizing the pharmacological effect of neuroplegic substances was regarded as a side effect secondary to the anticonvulsant effect and was determined by the "revolving roller" method [5]. The same method was used to study the side effects of Luminal and Diphenin, which in toxic doses induce ataxia, unsteady gait and disturbances of equilibrium and motor coordination [10]. The results of the experiments were compared. The median effective doses, or ED_{50}, required to produce the anticonvulsant effect and the TD_{50} required to evoke the side effects were determined by the method of Litchfield and Wilcoxon [8]. The TD_{50} was divided by the ED_{50} to obtain the "protective index" characterizing the therapeutic range of the preparations. D = 0.05 was used to

* Termed M. E. S. test in the English literature.

compute the authentic amounts of the ED_{50} and TD_{50}. The time at which the maximal anticonvulsant effect, or the peak of the effect, developed and the over-all duration of the effect were determined for each substance. This was done as follows: the experimental substance was administered to two groups of five mice each in doses equal to the ED_{50}; electrical stimulation was then administered by the method of electrical shock after 30 minutes to the first group of mice and after one hour to the second group. This procedure was repeated hourly until the complete disappearance of the anticonvulsant effect. In this way, the time at which the anticonvulsant effect developed, the time the effect became maximal, or the peak of the effect, and the total duration of the effect were determined. All the substances were analyzed by the electrical shock test according to the peak time of their anticonvulsant effects. The experimental animals used were male and female white mice weighing 24-29 g each.

We tested the following compounds: 10-(3-dimethylaminopropyl)-2-chlorphenothiazine hydrochloride (Aminazine), 10-(3-dimethylaminopropyl)-phenothiazine hydrochloride (Propazine), 10-(3-dimethylaminopropyl)-2-acetylphenothiazine maleate (Acepromazine), 10-(N-methylpiperidyl-3-methyl) phenothiazine acetate (Mepazine), 10-(N-methylpiperidyl-3-methyl)-2-chlorphenothiazine acetate (chlorine-containing Mepazine analog), 10-(2-diethylaminoethyl) phenothiazine hydrochloride (Dinezine), 10-(2-diethylaminoethyl)-2-chlorphenothiazine (chlorine-containing Dinezine analog), 10-[3-(I-β-hydroxyethylpiperazinyl-4) propyl]-2-chlorphenothiazine tartrate (Étaperazine), 10-[3-(I-methyl-4-piperazinyl)-propyl]-2-chlorphenothiazine hydrochloride (Compazine), 10-(β-diethylaminoproponyl)-2-chlorphenothiazine hydrochloride (Chloracizin) and the anticonvulsant substances Luminal (sodium salt) and Diphenin (5,5-diphenylhydantoin sodium). All the phenothiazine derivatives were intraperitoneally injected in aqueous solutions; Luminal was injected subcutaneously, and Diphenin was perorally administered through a probe in a 1% starch solution.

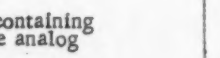
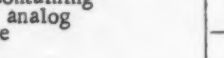
EXPERIMENTAL RESULTS

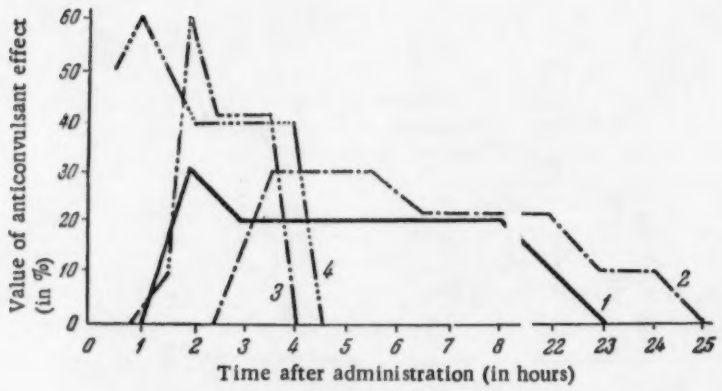
All ten of the experimental phenothiazine derivatives were found to be active anticonvulsants. The administration of these substances altered the typical course of the convulsive attack induced in white mice by electrical stimulation. The tonic extension phase, which was normally predominant in the picture of these attacks, was not observed, and the attack became clonic in character. Luminal and Diphenin manifested a similar anticonvulsant effect. For Aminazine, Propazine, Acepromazine and Mepazine, the ED_{50} was about the same (around 35 mg/kg); Aminazine and Acepromazine, however, caused such acute relaxation of the skeletal musculature that the animals became unable to move and acutely depressed, reacting weakly to external stimulations. The use of Mepazine in the same doses did not cause significant disturbances in the gait, posture or behavior of the animals. This indicates that the therapeutic value of a potential anticonvulsant agent cannot be defined by its ED_{50} alone; some criterion of the side effects, for which we used the TD_{50}, must be included in such calculations. The ratio TD_{50}/ED_{50} characterizes the therapeutic range of a substance's effect and is defined by the term "protective index." Other important criteria of an anticonvulsant substance's value are the speed with which its effect develops and the total duration of the effect. All these values are given in the table.

All the experimental phenothiazine derivatives can be divided into three groups of anticonvulsant activity: 1) the most active – Aminazine, Propazine, Acepromazine and Mepazine; 2) less active – Chloracizin, Dinezine and the chlorine-containing analogs of Dinezine and Mepazine; and 3) the least active – Étaperazine and Compazine. The picture is different, however, if the protective index rather than the ED_{50} is used as the criterion for comparative evaluation. The last column in the table shows only four compounds among the phenothiazine derivatives to have an index of more than unity, and of these compounds, only Mepazine and the chlorine-containing Dinezine analog possess a substantial therapeutic range. The protective indices of Propazine, Dinezine and the chlorine-containing Mepazine analog are close to unity, while those of Aminazine, Acepromazine, Étaperazine and Compazine are several times less than unity. According to their protective indices, Aminazine and Mepazine are not of the same value as potential anticonvulsant agents. Mepazine is more than 11 times as strong as Aminazine in this respect.

Another important criterion of the value of an anticonvulsant agent is the duration of its effect after a single administration. The graph shows these data for Aminazine, Mepazine, Luminal and Diphenin. We see that the effect of the phenothiazine derivatives is characterized by comparatively early manifestation, rapid attainment of its peak and a short total duration. The effect of Aminazine was less lasting than that of Mepazine. The chlorine-containing analogs of Mepazine and Dinezine produced the most lasting effects of the other penothiazine derivatives.

Anticonvulsant and Side Effects of Certain Phenothiazine Derivatives, Luminal and Diphenin

Substance	Chemical structure		Anticonvulsant activity as per electrical shock test			Side effect as per revolving roller test	Protective index
	R_1	R_2	ED_{50} (D = 0.05 used to compute authentic range)	peak of effect (in hours)	total duration of effect (hr)	TD_{50} (D = 0.05 used to compute authentic range)	$P.I. = \frac{TD_{50}}{ED_{50}}$
Aminazine	$-(CH_2)_3-N-(CH_3)_2$	Cl	34 (30.6—37.8)	2	2	6.4 (5.4—7.6)	0.2
Propazine	$-(CH_2)_3-N-(CH_3)_2$	H	32.8 (31.0—34.8)	1.5	2	40 (35.4—45.2)	1.2
Acepromazine	$-(CH_2)_3-N-(CH_3)_2$	$COCH_3$	32.5 (28.0—37.8)	0.5	1.5	3.9 (3.0—5.0)	0.1
Mepazine		H	35 (31.8—38.5)	1	3.5	82 (74—91)	2.3
Chlorine-containing Mepazine analog		Cl	79.0 (71.1—87.6)	1.5	4	77.0 (68.1—87.0)	0.99
Dinezine	$-(CH_2)_2-N-(C_2H_5)_2$	H	66.2 (58.6—74.9)	0.5	0.5	76.0 (59.4—97.3)	1.1
Chlorine-containing Dinezine analog	$-(CH_2)_2-N-(C_2H_5)_2$	Cl	70.0 (65.4—74.9)	2	3.5	102.5 (93.3—112.9)	1.5
Etaperazine		Cl	100.0 (91.8—108.6)	2	1.5	9.0 (8.2—9.9)	0.1
Compazine		Cl	114.0 (106.6—122.0)	1	1	18.6 (16.0—21.6)	0.16
Chloracizin	$-CO-(CH_2)_2-N-(CH_3)_2$	Cl	56.0 (49.1—63.8)	0.5	2.5	—	—
Luminal-Na	5-ethyl-5'-phenylbarbiturate (Na salt)		36.8 (33.2—40.8)	2	20	98.0 (86.8—110.6)	2.7
Diphenin	5,5-diphenylhydantoin		20.0 (17.4—23.0)	3.5	20.5	358.0 (317.0—405.0)	18.0



Duration and peak of anticonvulsant effect of neuroplegic and antiepileptic substances. 1) Luminal; 2) Diphenin; 3) Aminazine; 4) Mepazine.

It was interesting to compare the anticonvulsant properties and side effects of the phenothiazine derivatives with those of Luminal and Diphenin. The data for the latter two substances can be found at the bottom of the table. When the phenothiazine derivatives with the most active anticonvulsant effect were compared with these typical antiepileptic agents, Aminazine, Propazine, Acepromazine and Mepazine were found to be just as active as Luminal, although their effect was less durable; only Mepazine approximated Luminal, however, in value of protective index. Further, both Mepazine and Luminal were considerably less active than Diphenin.

As to the relationship between the chemical structure and pharmacological effect of the experimental substances, it is evident that the anticonvulsant activity of three compounds having carbon in the second position of the phenothiazine ring, but with different R_2 radicals (Propazine, Aminazine and Acepromazine), is approximately the same, while the central relaxant effect of the chlorine-containing analog (Aminazine) is much stronger than that of the nonsubstituted compound (Propazine), and the same effect is stronger still in the case of the acetyl analog (Acepromazine). Other effects (adrenolytic effect, potentialization of narcotic substances) of these substances are known to be strengthened by the substitution of a chlorine atom or acetyl radical [1] for the hydrogen atom, with carbon in the second position. It was also demonstrated in [1] that the ability of Mepazine to potentialize thiopental sleep is approximately half as great as that of its analog, although, as our table shows, the anticonvulsant effect of Mepazine is about twice as strong as that of its analog. On the basis of these data, one can propose that the anticonvulsant effect of neuroplegic substances should be differentiated from their general "central" effect. The mechanism of this effect, however, has not yet been sufficiently investigated.

SUMMARY

Ten phenothiazine derivatives were tested on mice for anticonvulsant potency at the time of their peak effect by the maximal electroshock seizure test (M. E. S.). Two clinically employed antiepileptic drugs – Luminal and diphenylhydantoin – were studied by the same technique. Their side effect (neurological toxicity) was investigated by the "rolling roller" method. All substances were found to abolish the tonic extensor phase of the maximal seizure. ED_{50}, TD_{50} and protective indices (P. I.) for all drugs have been determined. Diphenylhydantoin, Luminal and Mepazine had the highest P. I.; protective indices of Propazine, Dinezine, chlorsubstituted analogs of Mepazine and Dinezine approached 1. Aminazine, Acepromazine, Étaperazine and Compazine were effective only in toxic doses. The suggestion is made that anticonvulsive action of phenothiazine derivatives differed from their general "central" effect.

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DIFFERENTIATION OF THE DIFFERENT EFFECTS INDUCED BY ANTICHOLINESTERASE AGENTS

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Earlier, we discovered that proserine, which is regarded by most authors as an anticholinesterase agent only, affects a frog's isolated skeletal muscles in different ways, depending on the concentration: 1) the use of comparatively low concentrations ($1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$) causes repeated contractions; 2) the use of higher concentrations ($2 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$) is attended by the disappearance of these contractions; and 3) still higher concentrations induce contracture of the muscle [1, 2].

The first type of reaction has also been observed by N. A. Kozlov and M. Ya. Mikhel'son [3]; G. A. Panosyan [4] has reproduced all three variants of the proserine reaction (he discovered the first two variants, using the anticholinesterase agent phosphacol [diethyl p-nitrophenyl phosphate]).

The purpose of this work was to determine how the different variants of the skeletal muscle reaction would develop under the influence of the anticholinesterase agent Tensilon (edrophonium; 3-hydroxyphenyldimethylethylammonium chloride)[N-ethyl-N-(m-hydroxyphenyl)-N,N-dimethylammonium bromide].

We selected this preparation because it acts primarily on the skeletal muscles and has almost none of the muscarine effects characteristic of other anticholinesterase agents, proserine included [8, 9, et al.]. Tensilon was recommended for the treatment of myasthenia (myasthenia gravis) and has been found helpful in the diagnosis of this disease; it is inefficient in the treatment of this condition, however, because of the brevity of its effect. The brevity of Tensilon's effect can be explained by the instability of the complex formed, i.e., the enzyme cholinesterase + the inhibitor Tensilon. This proposition is in accord with Wilson's data [10]; this author, studying the association and dissociation rate of cholinesterase and Tensilon or proserine, established that the rate of cholinesterase association with the two substances depends on the concentration of the latter in the environment. In concentrations inducing 90% inhibition of cholinesterase activity, proserine causes 45% inhibition after one minute, Tensilon the same after only three seconds. The dissociation time of 50% of the cholinesterase + proserine complex is about seven minutes, that for the complex with Tensilon, less than 0.5 second.

EXPERIMENTAL METHOD

The experiments were performed on a frog's (Rana temporaria) isolated rectus abdominis muscle. The sartorius muscle was used in a few experiments. The experimental muscle was subjected to the action of the experimental substances one hour after isolation. The contractions which developed were recorded on a kymograph. Fifteen experiments were performed under these conditions at different intervals after denervation of the muscle (see method [2]). We used Roche Tensilon chloride, Schuhardt curare and proserine of Soviet manufacture in the experiments.

EXPERIMENTAL RESULTS

The first variant described above of the reaction which we observed earlier under the influence of proserine was reproduced in the experiments with Tensilon. In concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$, Tensilon induced repeated contractions characterized by rapid development and relaxation (Fig. 1). Analogous contractions were observed in the case of the sartorius muscle under the influence of the same concentrations of Tensilon. Tensilon's effect was preceded by a latent period lasting 2-25 minutes, but of shorter duration than that observed in the experiments with proserine. The duration of the latent period became longer as the concentration of substance decreased. The contractile reaction induced by Tensilon in our experiments lasted 15-40 minutes (the analogous proserine reaction lasted more than an hour).

Therefore, this variant of the contractile reaction is characterized by a more rapid development and a shorter duration when induced by Tensilon. This reaction could be intensified by proserine in low concentrations ($1 \cdot 10^{-6}$) and depressed by curare ($1 \cdot 10^{-5}$ to $5 \cdot 10^{-6}$). It gradually diminished with denervation and disappeared completely by about the 14th day after the nerves were transected (15-16 or more days after denervation, we observed a slowly developing contracture under the influence of Tensilon in concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$, instead of repeated, brief contractions).

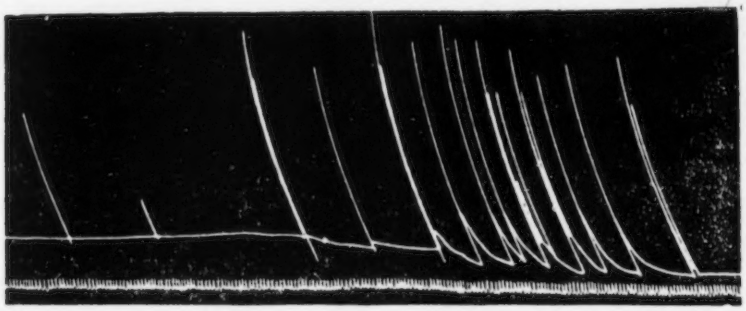

Fig. 1. Series of contractions of frog's rectus abdominis muscle induced by 1: 600,000 Tensilon. Time indicated in 5-second marks.

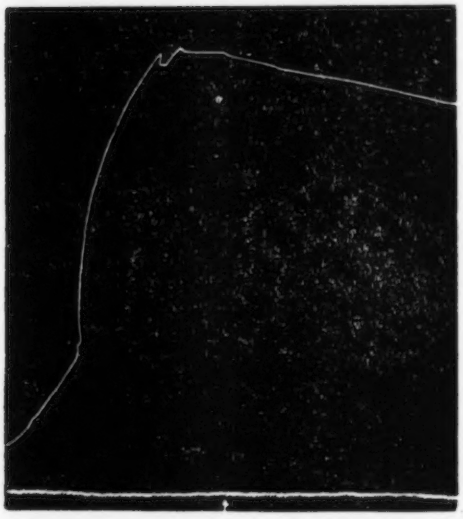

Fig. 2. Contracture of frog's rectus abdominis muscle induced by 1: 20,000 Tensilon (reduced $1\frac{1}{2}$ times). Arrow denotes addition of 1: 100,000 curare.

If the muscle was preliminarily treated with proserine in a concentration of $2 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$, it reacted to Tensilon by contracture. The repeated brief contractions did not appear in this case.

Higher concentrations of Tensilon ($1{,}6 \cdot 10^{-5}$ to $2 \cdot 10^{-5}$) caused a rapidly developing contracture type of contraction which was very slow to relax, although we observed on this background separate, low-amplitude contractions with rapid relaxation. Still higher concentrations of Tensilon ($3{,}3 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$) caused in all cases the rapid development of considerable contracture. This contracture was somewhat reduced by curare ($1 \cdot 10^{-5}$ to $2 \cdot 10^{-5}$), as can be seen from Fig. 2.

The data presented indicate that Tensilon reacts with the cholinergic system of a skeletal muscle to a greater degree than proserine, possibly with the actual contractile structure of the muscle as well. As well as the experiments using myographic recording, we also conducted experiments on the same muscles using electromyographic recording. According to the data obtained in these experiments, proserine and Tensilon,

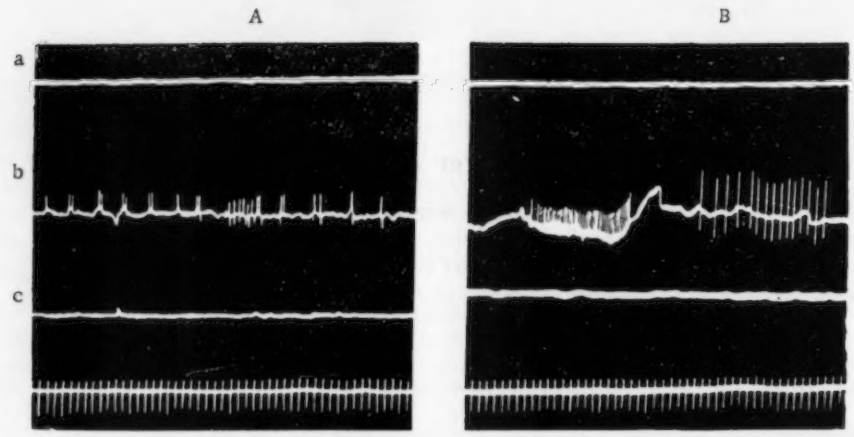

Fig. 3. Effect of proserine on electrical activity of frog's rectus abdominis (A) and sartorius (B) muscles. a) Absence of electrical activity before treatment of muscle with proserine; b) appearance of potentials under the influence of 1:200,000 proserine; c) disappearance of potentials under the influence of 1:25,000 proserine. Time shown in 0.1-second marks.

in concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$, promote the development of bioelectric potentials and increase the amplitude, frequency and duration of the spontaneously developing potentials; Tensilon's effect, however, is less lasting than that of proserine.

In higher concentrations ($2 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$), proserine reduced or completely inhibited the electrical activity of the muscles (Fig. 3). Tensilon was not tested in these concentrations.*

The results of the experiments using electromyography are in accord with the literature data. Fatt and Katz [6, 7] using frog's muscles, and Boyd and Martin [5], using muscles of warm-blooded animals, have shown that low concentrations of proserine (neostigmine – $5 \cdot 10^{-7}$) can be used to increase the amplitude and duration of the miniature potentials of end plates; Boyd and Martin further observed the reduction of these potentials with the use of higher concentrations of proserine (the authors do not state which concentrations or given any explanation for the results obtained).

Our earlier observations that the reactivity of frog's skeletal muscles to anticholinesterase agents varies considerably with the seasons was confirmed in this work. The greatest reactivity was observed during the winter season; the least, during the autumn and spring. The reaction is variable during the summer months.

SUMMARY

The effect of two anticholinesterasic substances – proserine and Tensilon – were compared in experiments on isolated frog muscles.

Three variants of reaction are provoked by eserine depending on its concentration: 1) repeated rapidly developing contractions of short duration; 2) disappearance of these contractions in using large concentrations; 3) contracture, when even higher concentrations are used.

Only two reactions were seen in testing Tensilon; they corresponded to the first and the third variants of proserine reaction. The first variant provoked by proserine and Tensilon is prevented by curare, denervation and sufficiently high concentrations of proserine (1:50 – 20,000). It is attributed to the anticholinesterasic properties of these preparations. The contracture variant in response to proserine and Tensilon application is explained by a combination of acetylcholine component (removed by curare) with a direct action of the mentioned anticholinesterasic substances on the muscle, which is not eliminated under the effect of curare. Proserine

* I wish to express my deep gratitude to Prof. D. G. Kvasov for making available to me the two-channel cathode-ray oscilloscope apparatus of the laboratories of the State Institute for Graduate Medical Studies and to A. P. Marevskaya for her help in the work.

and Tensilon in the concentrations of $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$ promote the appearance of muscle biopotentials. In higher concentrations (1 : 50 – 20,000) proserine depresses the electric activity provoked by its lower concentrations. The mechanism of this effect is still obscure.

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SOME DATA ON ESERINE'S EFFECT ON THE CEREBRAL CORTEX OF WHITE RATS

COMMUNICATION 1. THE EFFECT OF ESERINE ON CONDITIONED REFLEX ACTIVITY

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Eserine's effect on the functional activity of the cerebral cortex has not been adequately investigated [6, 9, 14, 18, 19, et al.], and the data obtained with the help of the conditioned reflex method do not concern the effect of eserine itself on the conditioned reflex activity of animals so much as the antagonism between eserine's effect and that of various cholinolytic substances [10, 16]. As for morphological research, the only relevant data we could find in the literature were those of S. A. Sarkisov and T. M. Mokhova [11] regarding the structural changes in the neurons and interneuronal synapses of rats given eserine. This work demonstrated that the change in the electrical activity of the cortices (motor and visual) effected by convulsant doses of eserine is attended by pathological changes in the nerve cells and their processes. This brings up the question of whether eserine only affects the condition of neurons and interneuronal synapses when used in convulsant doses, or whether smaller doses of the drug can have a similar effect; the question of histochemical changes is also interesting, and has not been studied at all.

The important role of ribonucleic acid (RNA) in the functional activity of the nerve cells is becoming more and more apparent. Most authors espouse the opinion of Casperson regarding the participation of RNA in the synthesis of proteins. Many investigations have demonstrated the link between the functional condition of neurons and their RNA content [2, 3, 4, 7, 8, 12, 13, 15, 16, 18, et al.].

The above formed the basis for our study of how different doses of eserine affect the dynamics of conditioned reflex activity in rats and the condition of the neurons (their morphology and RNA content) and structure of the interneuronal synapses in the cerebral cortex of rats.

EXPERIMENTAL METHOD

The investigation was performed on 35 male white rats weighing 200-300 g each, kept in a vivarium and fed the usual diet. Stable positive motor-food reflexes were developed in the animals over a period of $1\frac{1}{2}$ months by L. I. Kotlyarevskii's method [5]. The conditioned stimulus used was a bell of average force, the total effect of which lasted ten seconds. Seven combinations were given daily at intervals of 30-40 seconds. The conditioned reflex was considered to be developed if the rat responded correctly to the conditioned stimulus without reinforcement in every case or in five out of seven cases. Our purpose in this work did not require us to determine

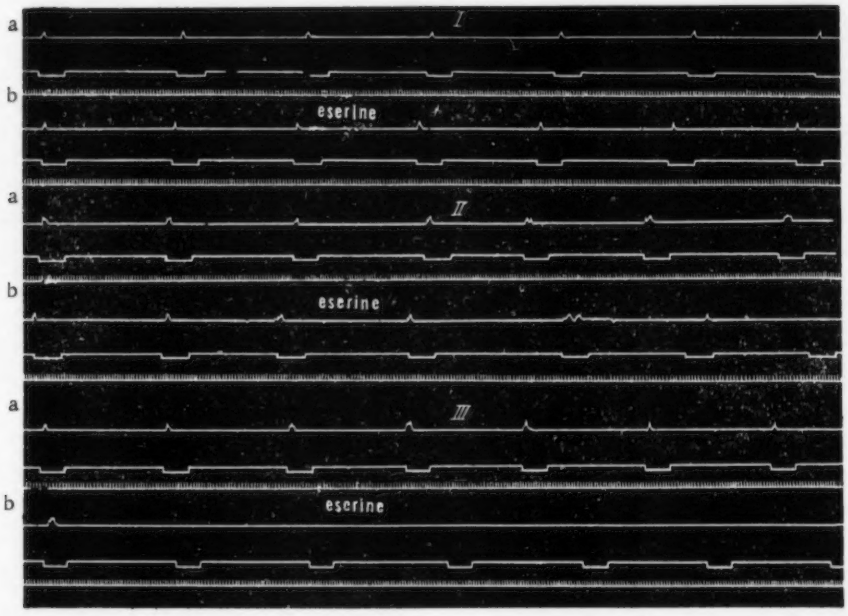
the animals' type of higher nervous activity, but the animals chosen for the experiments were those in which conditioned reflexes were developed by approximately equal numbers of combinations. Conditioned reflex activity was evaluated according to the duration of the latent period of the reflexes and to the force of the motor reaction.

The experimental animals were divided into three series. Eserine was subcutaneously injected 50-60 minutes before the animal was put into the chamber in a dose of 0.01 mg per 100 g body weight in the first series of animals, in a dose of 0.02 mg per 100 g body weight in the second and in a dose of 0.1 mg per 100 g body weight in the third series. The control animals were injected at the same times with the same doses of a saline solution.

EXPERIMENTAL RESULTS

The investigation showed that the administration of salt did not, as a rule, change the conditioned reflex activity of the rats.

In a dose of 0.01 mg per 100 g (see figure, I, b and Table 1), eserine shortened the latent period of the conditioned reflexes and caused some increase in the force of the motor reaction (rats No. 2 and 9). In several cases, single (rats No. 23 and 25) or repeated (rat No. 28) intersignal runs could be observed, although, in these cases, the administration of eserine did not cause change in the force of the motor reaction. The duration of the latent period and the force of the motor reaction were normal on the days following the injection of this dose of eserine.



Change in conditioned reflex activity of white rats effected by eserine. a) Before eserine injection; I, b) eserine injected in a dose of 0.01 mg per 100 g animal weight; II, b) eserine injected in a dose of 0.02 mg per 100 g weight; III, b) eserine injected in a dose of 0.1 mg per 100 mg weight.

When eserine was administered in a dose of 0.02 mg per 100g weight (see figure, II, b and Table 2), a stable change in the length of the latent period was observed in most of the animals; the latent period was usually shorter at the beginning of the experiment than it was before the eserine injection, but became longer at the end of the experiment. In some of the animals (rats No. 6 and 16), the force of the motor reaction was greater during the first 1-4 combinations in the experiment than before the eserine injection, but gradually decreased towards the end of the experiment; in other animals, however, the eserine injection either did not change

TABLE 1

Effect of Eserine Administered in a Dose of 0.01 mg per 100 g Weight on the Conditioned Reflex Activity of White Rats

Rat No.	Body wt. (in g)	Experiment date	Experimental conditions	Combination No.	Duration of latent period (in seconds)	Force of motor reaction (in mm)	Remarks
9	260	Aug. 18, 1958	Eserine not administered	237	2	6	Food eaten
				238	2	6	" "
				239	2	6	" "
				240	2	6	" "
				241	2.5	5	" "
				242	2	6	" "
				243	1.5	7	" "
9		Aug. 19, 1958	Eserine injected 50 minutes before experiment	244	1.5	6.5	Food eaten
				245	1	6.5	" "
				246	1	6.5	" "
				247	1	6.5	" "
				248	1.5	6	" "
				249	2	5.5	" "
				250	1.5	6	" "
25	280	March 11, 1959	Eserine not administered	210	2.5	7	Food eaten
				211	2	7	" "
				212	2.5	7	" "
				213	2.5	7	" "
				214	2	7.5	" "
				215	2.5	7	" "
				216	2.5	6	" "
25		March 12, 1959	Eserine injected 60 minutes before experiment	217	2 intersignal	7	Food eaten
				218	2	7	" "
				219	1.5	7	" "
				220	2	7	" "
				221	1.5	7	" "
				222	2	7	" "
				223	2	7	" "
28	250	March 24, 1959	Eserine not administered	230	2.5	5.5	Food eaten
				231	2.5	5.5	" "
				232	2.5	5	" "
				233	2.5	5.5	" "
				234	2.5	5.5	" "
				235	2.5	5.5	" "
				236	2	6	" "
28		March 25, 1959	Eserine injected 50 minutes before experiment	237	2 intersignal	5.5	Food eaten
				238	2 intersignal	5.5	" "
				239	2 intersignal	5.5	" "
				240	3 intersignal	6	" "
				241	2 intersignal	6	" "
				242	1.5	5.5	" "
				243	1.5	6	" "

TABLE 2

Effect of Eserine Administered in a Dose of 0.02 mg per 100 g Weight on the Conditioned Reflex Activity of White Rats

Rat No.	Body wt. (in g)	Experiment date	Experimental conditions	Combination No.	Duration of latent period (in seconds)	Force of motor reaction (in mm)	Remarks
16	240	Aug. 22, 1958	Eserine not administered	229	2	6	Food eaten
				230	2	6	" "
				231	1.5	6	" "
				232	2	6.5	" "
				233	2	6	" "
				234	2	6	" "
				235	2.5	5	" "
16		Aug. 23, 1958	Eserine injected 50 minutes before experiment	236	0.5	7.5	Food eaten
				237	1.5	7	" "
				238	1.5	7	" "
				239	0.5	7	" "
				240	6	6	" "
				241	8	5	" "
				242	no reaction	—	Did not approach
24	260	Mar. 11, 1959	Eserine not administered	202	3.5	8	Food eaten
				203	2	8	" "
				204	2	8	" "
				205	2	8	" "
				206	1	8	" "
				207	2	7	" "
				208	2	7	" "
24		Mar. 12, 1959	Eserine injected 60 minutes before experiment	209	1	7	Food eaten
				210	3	6	" "
				211	3	6.5	" "
				212	3.5	6	" "
				213	4	6	" "
				214	5	6	" "
				215	4.5	6	Not eaten
29	260	Mar. 24, 1959	Eserine not administered	190	2	5.5	Food eaten
				191	2	5	" "
				192	2	5,5	" "
				193	1	6	" "
				194	2	5.5	" "
				195	2	5.5	" "
				196	3.5	5.5	" "
29		Mar. 25, 1959	Eserine injected 50 minutes before experiment	197	1 intersignal	6	Food eaten
				198	2	5	" "
				199	2	5	" "
				200	1.5	5	" "
				201	2.5	5.5	" "
				202	4	5	" "
				203	5	5	" "

(rats No. 14 and 26) or reduced (rats No. 24 and 29) the force of the motor reaction. The motor reaction to the positive conditioned stimulus was sometimes observed to disappear at the end of the experiment (rats No. 6 and 26). The duration of the latent period and the force of the motor reaction were, as a rule, restored on the days following the experiments with eserine. The repeated administration of eserine caused a more acute disturbance of the rats' conditioned reflex activity; the latent period of the conditioned reflexes increased to such an extent

TABLE 3

Effect of Eserine Administered in a Dose of 0,1 mg per 100 g Weight on the Conditioned Reflex Activity of White Rats

Rat No.	Body wt. (in g)	Experiment date	Experimental conditions	Combination No.	Duration of latent period (in seconds)	Force of motor reaction (in mm)	Remarks
21	240	Aug. 22, 1958	Eserine not administered	233	2	7	Food eaten
				234	2	7	" "
				235	2	6	" "
				236	2	7	" "
				237	2	7	" "
				238	2	7	" "
				239	2	6	" "
21		Aug. 23, 1958	Eserine injected	240	4	6	Abandoned the food
				241	no reaction	—	Did not approach
				242	" "	—	" " "
				243	" "	—	" " "
				244	" "	—	" " "
				245	" "	—	" " "
				246	" "	—	—
27	300	Mar. 11, 1959	Eserine not administered	215	2	8	Food eaten
				216	intersignal 1 intersignal	7	" "
				217	2	7	" "
				218	2	7	" "
				219	2	7	" "
				220	2.5	7	" "
				221	2	7	" "
27		Mar. 12, 1959	Eserine injected 60 minutes before experiment	222	2,5	5.5	Food eaten
				223	4	7	" "
				224	8	6	" "
				225	jerkings	—	Did not open door
				226	7	6	Food eaten
				227	8	4	" "
				228	8.5	4.5	Did not eat
30	240	Mar. 24, 1959	Eserine not administered	199	1.5	6	Food eaten
				200	3	6	" "
				201	2	6	" "
				202	2	5	" "
				203	2	5.5	" "
				204	2	6	" "
				205	2	6	" "
30		Mar. 25, 1959	Eserine injected 50 minutes before experiment	206	4	5	Did not take the food
				207	no reaction	—	Did not approach
				208	23	4.5	Did not take the food
				209	10	5	Food eaten
				210	no reaction	—	Did not approach
				211	the same	—	" " "
				212	" "	—	" " "

that the motor reaction to the positive conditioned stimulus often disappeared. The unconditioned food reflex was retained, however; if food were placed on the floor of the chamber, the rat would eat it.

In a dose of 0.1 mg per 100 g body weight, eserine induced the development of a convulsive attack 13-18 minutes after the subcutaneous injection of the preparation. After the attack (see figure, III, b and Table 3), we observed the animals' conditioned reflex activity to be acutely disturbed; the latent period of the conditioned reflexes was so prolonged (rat No. 27) that the motor reaction to the positive conditioned stimulus often disappeared (rats No. 21 and 23). Although there was a considerable increase in the duration of the latent period, the force of the motor reaction varied; it either decreased (rats No. 21, 27 and 30), remained as before the eserine injection (rat No. 8) or even increased (rat No. 7).

Repeated administration of eserine in convulsant doses usually caused a more acute disturbance of the conditioned reflexes.

The type of change in the duration of the conditioned reflex latent period induced by the administration of the different doses of eserine to rats indicates that, in a dose as low as 0.01 mg per 100 g body weight, eserine affects the interneuron transmission of impulses, as shown by the shortening of the latent period of food-motor conditioned reflexes. When the dose is increased, eserine's effect disturbs the transmission of nerve impulses in the interneuronal synapses, as indirectly expressed by prolongation of the latent period of the reflexes up to the complete disappearance of the motor reaction.

The facilitating effect of eserine in small doses may be associated with the exaltation phase of this substance's effect discovered by L. S. Lanskaya [6] in experiments with mice given physiological doses of eserine (0.08-0.1 γ per 1 g weight).

SUMMARY

The study of the effect of eserine on the conditioned reflexes of white rats has demonstrated that its various doses exert a different effect on the conditioned reflex activity. In a dose of 0.01 mg per 100 g of body weight eserine reduces the latent period of the food-motor conditioned reflexes. Higher doses of eserine disturb the nerve impulse transmission in the interneuronal synapses; this is manifested in a considerable prolongation of the reflex latent period up to complete disappearance of the motor reaction to the positive conditioned stimulus.

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EFFECT OF RESERPINE ON THE ENTEROCHROMAFFIN CELLS OF THE SMALL INTESTINE

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At the end of the last century, N. K. Kul'chitskii (1897) described the enterochromaffin cells of the mucous membrane of the digestive tract. These cells have recently attracted the particular attention of researchers both here and abroad. Not long ago, Erspamer and Asero [6] isolated serotonin (5-hydroxytryptamine), which influences the vascular tonus [5], from a carcinoid tumor of these cells.

Numerous investigations have recently been published concerned with serotonin's effect on various organs and tissues, its distribution in the different systems of the organism and the search for serotonin antagonists. The data indicating serotonin's role in increasing arterial blood pressure are especially important [5]. The histochemical investigations of Benditt and Wonj [4] established that enterochromaffin cells contain about 1% serotonin. Under the influence of reserpine [7], serotonin disappears from the enterochromaffin cells [10] and is decomposed by monoamine oxidase. In their article [3], A. I. Yakovleva and N. G. Shakhnazorova showed that, depending on the condition of the animals (starved and not starved) and on the method of reserpine administration, reserpine caused the argyophil granules in enterochromaffin cells to disappear. These works investigated the effect of a single administration of reserpine in large doses on the morphology of enterochromaffin cells. We were unable to find in the literature available information on the effect of reserpine, systematically administered for a prolonged period, on the enterochromaffin cells. Nor has reserpine's effect on the number of enterochromaffin cells yet been investigated.

We therefore decided to conduct a special study under conditions of a chronic experiment to investigate the effect of repeated administrations of reserpine in comparatively small doses on the enterochromaffin apparatus of the small intestine, specifically, that of the duodenal mucous membrane. This portion of the intestine was selected for study because, in guinea pigs, it is known to contain the greatest number of enterochromaffin cells and the extremely receptor-rich zone of the small intestine; here the most important digestive juices are secreted, and the reflexes inducing increase of arterial pressure during alimentary stimulation originate from here.

EXPERIMENTAL METHODS

The experiments were performed on eight guinea pigs. The experimental animals were divided into four groups. The guinea pigs of the first, or control, group did not receive reserpine. Daily peroral administrations of reserpine were given to the three experimental groups, in a dose of 0.05 mg for the second group, 0.125 mg for the third group and 0.25 mg for the fourth group. After receiving reserpine for a month, the animals were decapitated, and various parts of the duodenum were taken for histological examination. The material was fixed with Lison's mixture and with a 10% formalin solution. The paraffin blocks were cut into sections 4-5 microns thick. The acid diazo reaction and Held's hematoxylin stain were used to demonstrate the enterochromaffin cells.

EXPERIMENTAL RESULTS

Considerable numbers of enterochromaffin cells were found in the control animals along the whole length of the duodenum. These cells were arranged singly or in groups and usually located in the epithelial lining of the crypts, occasionally in the substance of the Brunner's glands and also in the epithelial sheath of the villi (Fig. 1). The bodies of the cells were irregularly shaped. In the protoplasm of these cells, the acid diazo reaction demonstrated profuse, reddish-brown granulation. In some of the cells, the granulation was more or less evenly distributed. Cells with basally situated granulation were, however, observed (Fig. 2). Most of the enterochromaffin cells were found on the bottom of the crypts or on adjacent areas. Much more rarely, enterochromaffin cells were found in the substance of the epithelial sheath covering the lateral surfaces of the villi. In these cells, the granulation was somewhat finer, less abundant and basally situated.

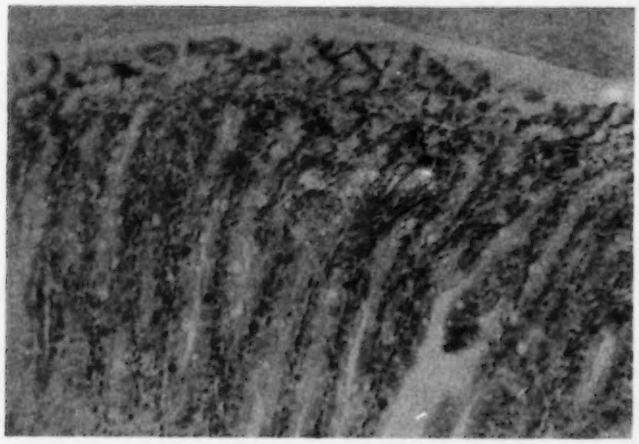

Fig. 1. Duodenal mucous membrane of control guinea pig. Many enterochromaffin cells (the dark cells) are apparent on the bottom of the intestinal crypts, as well as in the epithelial sheath of the main villi. Acid diazo reaction with hematoxylin afterstain. Magnification 200 ×.

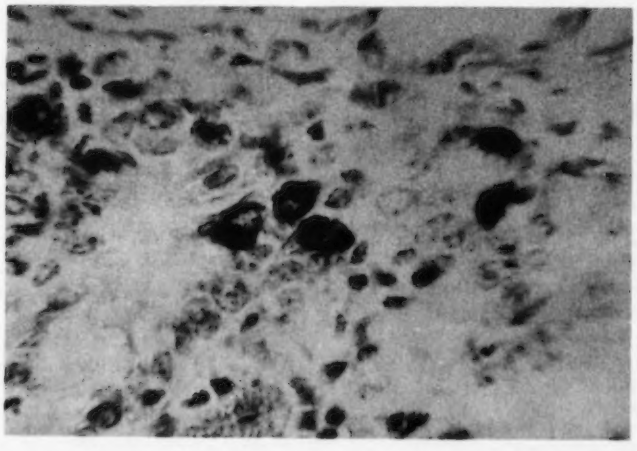

Fig. 2. Duodenal mucous membrane of the same animal. Profuse granulation is seen filling the apical and basal portions of the cells. Acid diazo reaction with hematoxylin afterstain. Magnification 1000 ×.

TABLE 1

Change in Enterochromaffin Cells Effected by Reserpine

Reserpine dosage	Total number of enterochromaffin cells			
	M	M	t	D
Control (reserpine not administered)	171	23.61	–	–
0.05 mg per day	127	16.8	1.55	0.05
0.125 mg per day	96	9.2	3.0	0.01
0.25 mg per day	103	8.7	2.3	0.03

TABLE 2

Effect of Reserpine on Granulation Content of Enterochromaffin Cells

Reserpine dosage	Enterochromaffin cells with large number of granules		Enterochromaffin cells with small number of granules	
	number	%	number	%
Control (reserpine not administered)	107	62.5	64	37.5
0.05 mg per day	56	44.1	71	55.9
0.125 mg per day	33	34.4	63	65.6
0.25 mg per day	40	38.9	63	61.1

In the experimental animals, both the total number of enterochromaffin cells (Fig. 3) and the granulation content of the cells (Fig. 4) were found to be reduced under the influence of reserpine. We tabulated the number of enterochromaffin cells, dividing them into two groups: 1) the cells containing a large number of granules; 2) the cells containing a small number of granules. We computed the arithmetic mean (M), the square deviation (δ), the standard error (t) and the authenticity of the difference (D) between the different groups according to the Student-Fisher index.

Table 1 gives data showing the changes in the total number of enterochromaffin cells affected by different dosages of reserpine.

As Table 1 shows, the average number of enterochromaffin cells per 100 crypts was 171 in the control animals. In the animals which had received 0.05 mg reserpine daily for a month, the average number of enterochromaffin cells was reduced to 127 per 100 crypts. The decrease in the number of enterochromaffin cells observed after the administration of reserpine in these doses was statistically unreliable and did not exceed the control ($t = 1.55$, $D < 0.05$).

The changes were considerably more pronounced in the animals which had received 0.125 mg reserpine daily for a month than in the preceding experimental variant. In this case, the number of enterochromaffin cells decreased to 96 per 100 crypts. This decrease in the number of cells was found to be statistically authentic ($t = 3.0$, $D < 0.01$) as compared with the control. The number of enterochromaffin cells was also reduced in the animals which had received the 0.25 mg daily dose of reserpine (103 cells per 100 crypts). The difference between the control and the experiment was found to be statistically authentic in this case also ($t = 2.3$, $D < 0.03$).

Reserpine induced a change in the intimate structure of the cells as well as the decrease in their total number. The number of granules in the cells decreased, and the main mass of granulation moved into the apical part of the cell (see Fig. 1). The zone located under the nucleus of these cells was usually free of granules definable by the acid diazo reaction or Held's hematoxylin. The larger doses of reserpine caused the enterochromaffin cells to lose their definite outlines and to become smaller. Single tiny granules could be barely seen against the clear background of the protoplasm. A typical effect of reserpine in these cases was a marked change in the correlation between the enterochromaffin cells which contained numerous granules in the protoplasm and those with a low granule content. These data are given in Table 2.

As Table 2 shows, 62.5% of the enterochromaffin cells had a high granule content and 37.5% contained a small number of granules (the ratio of the cells with a large number of granules to those with a small number constituted 2:1).

After the administration of reserpine in a daily dose of 0.05 mg, 44.1% of the cells contained a large number of granules, 55.9%, a small number, giving a ratio of 4:5.

Therefore, reserpine caused a marked decrease in the number of enterochromaffin cells with a high granulation content. This change was even more marked when reserpine had been administered in a daily dose of

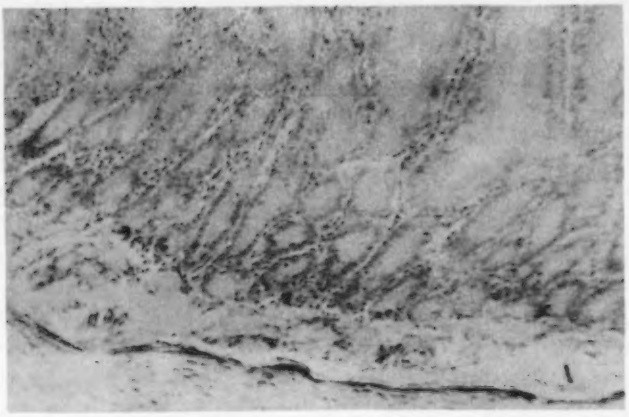

Fig. 3. Duodenal mucous membrane of guinea pig given reserpine in a dose of 0.25 mg a day. Sharp decrease in the content of enterochromaffin cells in the intestinal mucous membrane. Only 4-6 enterochromaffin cells evident in the entire field of vision. Most of the crypts devoid of enterochromaffin cells. Acid diazo reaction with hematoxylin afterstain. Magnification 200 ×.

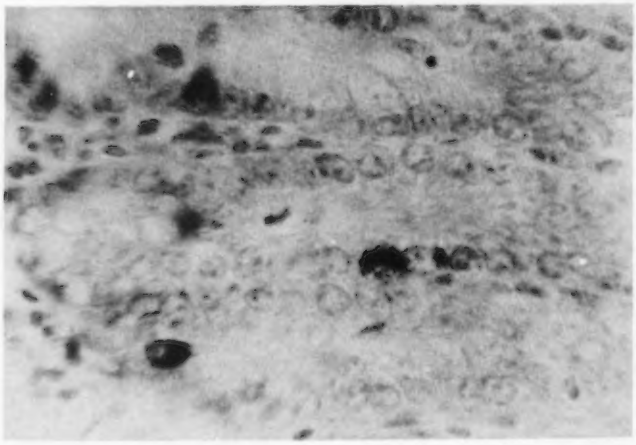

Fig. 4. Duodenal mucous membrane of the same animal. Reduced granulation content in enterochromaffin cells. Granulation has shifted to apical portion of cell. Acid diazo reaction with hematoxylin afterstain. Magnification 1000 ×.

0.125 mg. With this dose, the number of cells with a large number of granules decreased to 34.4%, and the number of cells containing few granules increased to 65.6%. Their ratio, then, was 1: 2. Finally, when reserpine was administered in doses of 0.25 mg a day, the cells with a large number of granules comprised 38.9%, while 61.1% contained a small number, i.e., the decrease in the granulation content of the cells is clear.

The observations we conducted, therefore, show that the administration of reserpine to the guinea pig organism for a month decreased the total number of enterochromaffin cells in the duodenum and either diminished the formation of granules in the protoplasm of enterochromaffin cells or caused the complete disappearance of these granules.

Since enterochromaffin cells produce serotonin, which definitely participates in the rise of arterial pressure, the decrease in arterial pressure which attends the prolonged administration of reserpine, both under normal conditions and under conditions of hypertonia, is probably due chiefly to the effect of reserpine on the enterochromaffin cells.

In our earlier investigations [1], we established that reserpine reduces both increased and normal blood pressure in experimental animals. The effect of reserpine perorally administered is only apparent after 3-5 days. The long duration of this preparation's latent effect is evidently due to its action on the systems by means of which a certain blood pressure level is maintained, possibly to its action on the enterochromaffin cells.

SUMMARY

To investigate the mechanism of the reserpine effect on the blood pressure an experiment was done on 8 guinea pigs with a corresponding control. Experimental animals were given reserpine in the doses of 0.05 mg, 0.125 mg and 0.25 mg. No reserpine was administered to control animals.

In a month after the reserpine administration was started the animals were sacrified and various portions of their duodenum were subjected to histological examination. The total number of enterochromaffin cells in the duodenum was found to be reduced; the formation of the granules in the protoplasm of chromaffin cells was diminished or these granules were totally lost.

A suggestion is made that the drop of arterial pressure occurred as a result of reserpine action upon the enterochromaffin cells of the small intestine, which produce serotonin.

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INHIBITION OF THE ANAPHYLACTIC REACTION BY THE CREATION OF A DYNAMIC STEREOTYPE

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(Presented by Academician V. N. Chernigovskii)
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The study of the defensive and regulatory activity of the central nervous system in immunological and allergic processes is one of immense importance to the elucidation of the physiological mechanisms of reactions in response to antigenic stimuli. Investigations by Soviet physiologists and immunologists [3, 6, 7, 8, 10] have established the presence of a connection between the functional state of the central nervous system and the activity of the apparatus of immunogenesis.

The regulation of the course of allergic reactions by the higher divisions of the brain, representing as it were the reverse side of immunological phenomena, has been inadequately studied from the point of view of the significance of these problems in the theory and practice of immunity. It would be of great interest to discover the role of a cortical dynamic stereotype, as a complex system of conditioned reflexes, in the reaction of the body to an antigenic stimulus.

The development of our knowledge of the patterns of activity and the functional properties of the higher divisions of the brain has led physiologists to regard the cortex as having a powerful influence on the realization of unconditioned acts. It has been shown, in particular [2, 5], that the reaction to unconditioned stimuli depends not only on the physiological properties of the actual stimulus but also on the general neurodynamic background, on the combination of excitation and inhibition to be found in the central nervous formations.

The importance of the initial neurodynamic background to the reactivity of the animal body was shown especially clearly by the work of Dolin and his collaborators during the study of the systemic character of the work of the brain, which was a further development of ideas put forward by Pavlov's school.

The formation of a dynamic stereotype in the activity of the brain is based on its ability to form and consolidate the functional integration of the centers when these are systematically bombarded with nervous impulses from the periphery. The source of the impulses for these centers is provided by the action on the body of an external stereotype of stimuli which, when applied repeatedly and uniformly, is fixed in the cerebral cortex, as a result of which a dynamic stereotype is produced. The characteristic properties of the stereotype are its inertia, its resistance to change and its ability to overcome "new situations and new stimuli."

It has now been established [1, 4, 5, 9, 11] that, after the production of a stable stereotype of conditioned reflexes, cortical influences may distort the reactions to different unconditioned stimuli of a chemical, physical and antigenic nature.

In the light of the foregoing remarks it appeared possible and necessary to make an experimental study of the physiological mechanisms of the participation of the cerebral cortex in the regulation of allergic reactions, using the anaphylactic reaction as an example.

EXPERIMENTAL METHOD

Experiments were carried out on 51 guinea pigs in the course of 1958-60.

On each successive day the experimental animals were fixed in a special stand, where, in indentical experimental conditions and in a strictly constant time and order of stereotype, they were systematically injected subcutaneously with isotonic sodium chloride solution, and then 15-30 minutes later food reinforcement (carrot) was given. All these manipulations were carried out by the same experimenter.

The animals were thus subjected to the action of an external stereotype of stimuli, incorporating a complex group of moderately strong tactile, visual, auditory, proprioceptive and chemical stimuli, accompanying the injection of sodium chloride and preceding the food reinforcement.

The daily stereotyped injections of physiological saline under constant experimental conditions lead to the formation and consolidation of conditioned reflex connections not only to the injection of physiological saline but also to the whole complex experimental situation. With such a stereotype of external stimuli there is created a functional integration of the excited and inhibited nervous centers, which is brought about by the formation of a dynamic structure of interconnected, complex physiological processes in the body as a whole.

The animals were sensitized by means of crude horse serum in dilutions of 1:1000 or 1:100, in a volume of 1 ml, given subcutaneously. The assaulting dose (1 ml) of protein stimulus was given in the form of a 40-80% solution of horse serum. The number of daily stereotyped injections of 1 ml of physiological saline varied from 28 to 40, and sensitizing dose of protein was given after 9-15 daily injections of physiological saline, while the assaulting dose was given after a further 18-25 days.

The degree of severity of the anaphylactic shock and the intensity of the reaction were noted as follows: ++++ lethal shock; +++ severe shock; ++ moderate shock; + slight shock; − general and marked inhibition.

EXPERIMENTAL RESULTS

In the first experiments, carried out on 11 guinea pigs to study the influence of systemic factors in the activity of the brain on the realization of the action of the antigenic stimulus, a dynamic stereotype was formed as described above. On the 28th day after the beginning of formation of the stereotype, the deliberate alteration of the leading component of the external stereotype of stimuli − replacement of physiological saline by an assaulting dose of protein (horse serum in a volume of 1 ml of a 40% solution) − led to the marked inhibition or weakening of the response reaction of the animal to the protein stimulus.

Of the five experimental animals (Nos. 1-5) only two (Nos. 3 and 4) developed anaphylactic shock in a mild or moderately severe form (Fig. 1a). The other three guinea pigs (Nos. 1, 2 and 5) reacted to the injection of horse serum by a state of general inhibition, accompanied by adynamia and by the loss of several unconditioned reflexes (feeding and orientational).

Of the six control animals, four (Nos. 7, 8, 9 and 11) developed a severe form of shock after injection of the assaulting dose of protein, and guinea pig No. 11 died from shock. Only two animals (Nos. 6 and 10) developed a mild or moderately severe form of anaphylactic shock after injection of horse serum (see Fig. 1a). It could be provisionally concluded from these pilot experiments that, by actively creating a definite trend in the course of the fundamental nervous processes by modifying the external stereotype of stimuli, the sensitivity of the animal to the protein stimulus could be significantly altered.

The further development of these experiments was connected with the necessity of studying the effect of systemic factors on both the process of development of sensitization and the efficacy of action of the assaulting dose. It was thus necessary to discover what dose of protein − sensitizing or assaulting − loses its specific action. We accordingly carried out a second series of experiments in two variants on 15 guinea pigs.

In one variant of the experiments (guinea pigs Nos. 12-16), before the establishment of a dynamic stereotype, the animals received an injection of the sensitizing dose of protein (1 ml in a dilution of 1:1000), after which we proceeded with the formation and consolidation of the cortical stereotype.

The subcutaneous injection of the assaulting dose of protein (1 ml of a 40% solution of horse serum) given on the 27th day after the daily stereotyped injections of physiological saline, did not lead to the obvious

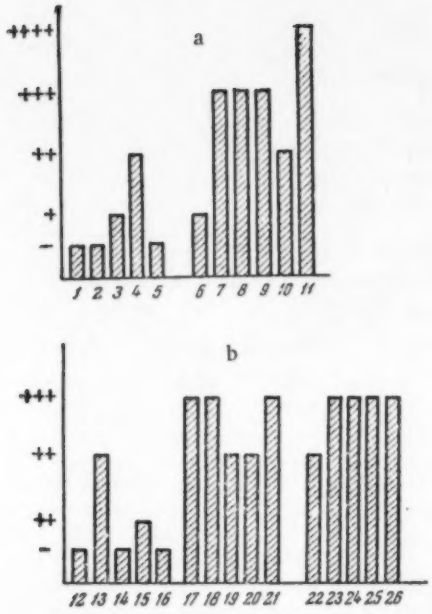

Fig. 1. The influence of systemic mechanisms in the activity of the brain on the realization of an antigenic stimulus in guinea pigs.

appearance of anaphylactic shock (Fig. 1b). It is evident that in guinea pigs Nos. 12, 14 and 16 anaphylactic shock did not develop at all, whereas in guinea pigs Nos. 13 and 15 a mild and moderately severe form of shock developed in response to the injection of horse serum. The results of this variant of the experiments were in good agreement with our original findings that it is possible in principle to inhibit an anaphylactic reaction by means of systemic mechanisms.

In the other variant of the experiments (animals Nos. 17-21) the sensitizing dose was injected, not before the formation and consolidation of the cortical stereotype as in the previous experiment, but after, which would enable us to investigate the problem of the arresting of the process of sensitization by means of systemic mechanisms in the activity of the brain.

In this series of experiments, after the formation of the dynamic stereotype, on the 27th day after daily stereotyped injections of physiological saline, the latter solution was deliberately replaced by 1 ml of sensitizing dose of protein in a dilution of 1:1000, and immediately afterwards the experiments were interrupted for 18 days. After this time had elapsed in the conditions of the stereotyped injections of physiological saline, the animals received a subcutaneous injection of 1 ml of a 40% solution of horse serum. The experiments showed that under these conditions all the guinea pigs developed anaphylactic shock in a moderately severe or severe form. In the control group of animals all the guinea pigs (Nos. 22-26) reacted to the injection of horse serum in the same dilution by the development of a severe or moderately severe form of anaphylactic shock.

The facts obtained from this series of experiments provided further evidence that the threshold of sensitivity of an animal to a protein stimulus may be essentially changed by a conditioned reflex method. At the same time it was found that it is easier to inhibit the reaction to the assaulting dose of protein than the reaction to the sensitizing dose of protein by means of the mechanisms of a dynamic stereotype. A similar conclusion emerges from an analysis of the available literature on the inhibition of the anaphylactic reaction by various types of agents [12, 13, 14]. Meanwhile the analysis of our own findings does not permit a final conclusion to be drawn. Other variants of experiments are required, and it is hoped to continue with these in the future.

The further analysis of this problem which we are considering led to the necessity of carrying out a series of experiments to study the stability and the physiological strength of the systemic mechanisms when they exerted their inhibitory influence on the mechanisms of the anaphylactic reaction. For this purpose we both increased the total number of stereotyped injections of physiological saline to 40 (instead of 26-28 in the previous experiments) and increased the intensity of the antigenic stimulus and the sensitizing dose (for sensitization we used serum in a dilution of 1:100; for the assaulting injection we gave 1 ml of a 40-80% solution of serum). The experiments were carried out on 15 guinea pigs. As controls we used two groups of guinea pigs (10 animals).

In the experimental animals we produced a stable stereotype of reactions to the subcutaneous injection of physiological saline (40 injections altogether), which was then replaced by the protein stimulus (the subcutaneous injection of 1 ml of a 40-80% solution of horse serum). The guinea pigs of the first control group were injected with horse serum in the same dilution. The guinea pigs of the second control group were given daily subcutaneous injections of physiological saline in a volume of 1 ml, but the experimental conditions were varied: the injections were given in different places, in different parts of the body and at different times and in a different order. By means of this modification of the experiments we were able to show experimentally how the variation of the conditions affects the formation of the stereotype and that physiological saline itself has no nonspecific desensitizing action.

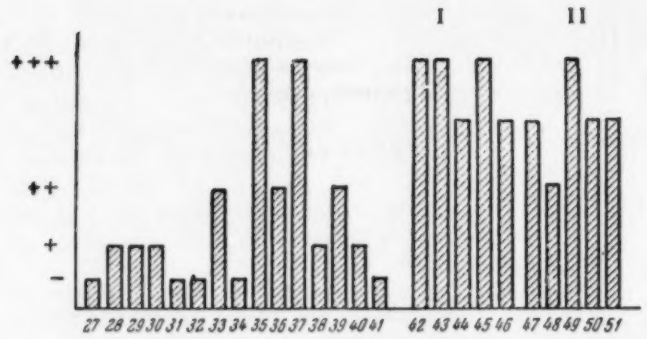

Fig. 2. Inhibition of the anaphylactic reaction by the mechanisms of a dynamic stereotype.

As shown in Fig. 2, experimental guinea pigs Nos. 27, 31, 32, 34 and 41 reacted to the subcutaneous injection of an assaulting dose of protein by a severe general inhibition, extending also the subcortical formations, manifesting itself as the loss of orientational and feeding unconditioned reflexes.

Guinea pigs Nos. 28, 29, 30, 38 and 40 developed a mild form of anaphylactic shock. Three guinea pigs (Nos. 33, 36 and 39) reacted to the injection of protein by anaphylactic shock of a moderate degree of severity. Only in guinea pigs Nos. 19 and 21 did a severe form of shock terminate in death.

In both control groups the injection of horse serum in the same dilution was followed by the development of severe manifestations of shock, terminating in individual cases in death.

It follows from the experiments described that the effect of inhibition of the anaphylactic reaction by the mechanisms of a dynamic stereotype is a function of two variables: the stability of the induced stereotype and the strength of the antigenic impulse. The depth of inhibition of the anaphylactic reaction increases with an increase in the stability of the stereotype and decreases with an increase in the strength of the antigenic impulse. Furthermore it is essential to have strict observance of the technical conditions of formation of the stereotype, as is illustrated by the results of the experiments on the animals of the second control group of the last series. It is important in principle to emphasize that the role of a dynamic stereotype in the modification of immunobiological reactivity is effective only when the animal is subjected to the action of threshold, i.e., of relatively weak, shock-producing doses of protein, and when the strength of the antigenic pathological stimulus is increased, the physiological mechanism of defense of which we consider the functional manifestation to be the dynamic stereotype becomes less effective.

It may be postulated that in all these experiments we are concerned with the formation of a complex conditioned reflex to the internal state of the animal, characterized by the pattern of the unconditioned reactions and of their underlying metabolic processes, themselves caused by the particular system of stereotyped stimuli, the first of which become conditioned stimuli setting in motion the chain of subsequent unconditioned reflex reactions.

This dynamic pattern of unconditioned processes, incorporating the reactions of many systems of the body and caused by a complex conditioned stimulus and increasing the resistance of the animal to pathogenic agents, may be designated a complex conditioned "reflex of nonspecific resistance."

By means of this method it is possible to judge the role of the normally functioning cerebral cortex in the course of immunological and allergic processes, and to ascertain the character of the cortical influence, its duration and its strength.

SUMMARY

It was established by experiments carried out on guinea pigs that it is possible to change the sensitivity of the body to antigenic stimulation by creating dynamic stereotype in the brain hemispheres. It was shown that it was possible to inhibit, to invert or to considerably diminish the anaphylactic shock by the systemic mechanisms. The extent of anaphylactic shock inhibition by the systemic mechanisms depends on the stability of dynamic

stereotype and the strength of the antigenic impulse. The mechanisms of dynamic stereotype are effective only in the action upon the organism of the threshold (i.e., the relatively weak) shock doses of the protein stimulus. In analyzing our own material and the data of other authors a suggestion is made that the dynamic sterotype is a functional manifestation of the physiological preventive measure against pathological actions.

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RESISTANCE OF THE SPECIFIC ANTIGEN OF HUMAN MALIGNANT TUMORS TO A HIGH TEMPERATURE

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The improvement of methods of detection of the specific antigens of human malignant tumors has made it possible to study the physicochemical properties of these antigens. The detailed study of the nature and the physicochemical properties of the specific antigens of malignant tumors is of great interest, but the facts at present known about this subject are extremely limited and controversial. This is because of the complexity of the antigenic structure of both normal and malignant tissue, and also because of the great technical difficulties in the way of their immunological differentiation.

Our method [3, 4, 5] of selective absorption has shown [2] that the specific antigen of human carcinoma is resistant to the action of formalin and glycerol, and does not lose its antigenic and immunogenic properties as a result of such treatment: the tissues of malignant tumors retain the ability to specifically absorb the corresponding antibodies from immune sera, and saline extracts of tissues treated with these substances are active in serological tests and cause antibody formation in rabbits.

The tumor antigens are also resistant to the action of low temperatures and to vacuum drying. In our researches we have constantly used antigens prepared from tumor tissues frozen at −20° or dried by the lyophilic method without thereby observing any decrease in their antigenic activity by comparison with untreated tissues.

Information in the literature regarding the action of a high temperature on the antigenic properties of tumors of man and animals is scanty and indicates merely the disappearance of the antigenic properties of the tumors on heating [1], or their thermostability [7-10 and others]. As has been pointed out above, the contradictory nature of the results obtained by different workers may be attributed to differences and imperfections in the methods used.

In the present research our object was to study the resistance of the specific antigens of human malignant tumors to a high temperature.

EXPERIMENTAL METHOD

The material used for this study was tissue from malignant tumors (metastases in the liver) of patients dying from cancer. As controls we used tissue from organs (liver, kidney, spleen, heart muscle, lung and brain) from the same patients not affected by tumors, and also spleen tissue from healthy persons killed in accidents.

As antigens we used saline extracts of the crude tissues and of tissues heated for 30 minutes at 100°. A suspension of finely minced tissue in physiological saline (1:10) was boiled, then ground again in a mortar and centrifuged. The supernatant fluid was used as antigen. Rabbits were immunized with antigens from the untreated and boiled tumor tissues, and also with antigen from the untreated spleen from the healthy individual.

TABLE 1

Comparative Study of Antigens from Various Human Tissues (untreated and boiled) by Means of the Complement Fixation Reaction

Specific serum	Dilution of serum	Antigens from tissues of patient P													
		untreated							boiled						
		malignant tumor	liver	kidney	spleen	heart muscle	lung	brain	malignant tumor	liver	kidney	spleen	heart muscle	lung	brain
No. 211 to untreated tumor tissue of patient P	1:40	++++	–	–	–	–	–	–	++++	–	–	–	–	–	–
	1:80	++++	–	–	–	–	–	–	++++	–	–	–	–	–	–
	1:160	+++	–	–	–	–	–	–	++	–	–	–	–	–	–
	1:320	+	–	–	–	–	–	–	+	–	–	–	–	–	–
No. 834 to untreated spleen tissue of a healthy person	1:40	–	–	–	++++	–	–	–	–	–	–	–	–	–	–
	1:80	–	–	–	+++	–	–	–	–	–	–	–	–	–	–
	1:160	–	–	–	+	–	–	–	–	–	–	–	–	–	–
	1:320	–	–	–	±	–	–	–	–	–	–	–	–	–	–

Conventional signs: ++++, +++, ++, +, ± various degrees of positive reaction; – negative reaction.

The method of obtaining antitumor sera and of freeing them from contaminating antibodies, and also the method of performance of the complement fixation and absorption reactions which we used in our experiments are described in our previous communications [3, 4].

EXPERIMENTAL RESULTS

In Table 1 we give one of the typical experimental records from the comparative study of antigens from untreated tissues and tissues subjected to boiling, taken from patient P (blood group A), who died from carcinoma of the stomach with metastases in the liver. It will be clear from Table 1 that the specific antitumor serum No. 211, obtained against untreated antigen from the tumor in patient P and free from contaminating antibodies, gave a positive complement fixation reaction not only with antigen from the untreated tumor but also with the extract of tumor tissue subjected to boiling. Antigens from the liver, kidney, spleen, heart muscle, lung and brain of the same patient, whether untreated or boiled, gave no reaction. This is evidence of the presence in the tumor tissue, both before and after heating, of a specific antigenic component which is absent from the investigated organs of the same patient. The organ-specific serum against spleen tissue, used as a control, reacted only with untreated antigen from spleen tissue and gave no reaction with spleen antigen heated for 30 minutes at 100°. It follows from the experiments that the antigen which is specific for human spleen is destroyed by boiling the tissue for 30 minutes, as was previously shown by the work of Kuznetsova [6].

TABLE 2

Investigation of Antigens from Tissues of Patient P in an Absorption Experiment

Specific serum	Tissue used for absorption of serum	Dilution of serum	Antigens from tissue of patient P			
			untreated		boiled	
			malignant tumor	spleen	malignant tumor	spleen
No. 211 to untreated tumor from patient P	—	1:40	++++	—	++++	—
		1:80	++++	—	++++	—
		1:160	+++	—	++	—
		1:320	++	—	±	—
	Boiled spleen from patient P	1:40	++++	—	++++	—
		1:80	++++	—	+++	—
		1:160	++	—	+	—
		1:320	±	—	±	—
	Boiled malignant tumor from patient P	1:40	—	—	—	—
		1:80	—	—	—	—
		1:160	—	—	—	—
		1:320	—	—	—	—
No. 834 to untreated spleen of a healthy person	—	1:40	—	++++	—	—
		1:80	—	+++	—	—
		1:160	—	++	—	—
		1:320	—	±	—	—

Conventional signs as in Table 1.

By means of the complement fixation reaction we thus demonstrated the resistance of the specific antigen of human carcinoma to heating to 100° for 30 minutes, in contrast to the behavior of organ-specific antigens, especially of the spleen, which lose their serological activity under these conditions.

The resistance of the tumor antigen to a high temperature was also confirmed by absorption experiments. The results of the experiments on the absorption of the specific antitumor serum No. 211 with tumor and spleen tissues from patient P, treated by boiling, are given in Table 2.

TABLE 3

Experiment of Absorption of Serum Obtained Against Antigen from a Tumor in Patient P and Heated to 100°

Serum	Tissue used for absorption of serum	Dilution of serum	Antigens from tissue of patient P											
			untreated					boiled						
			malignant tumor	spleen	heart muscle	lung	brain	malignant tumor	spleen	heart muscle	lung	brain	Antigen from spleen of a healthy person
No. 211 to untreated tumor from patient P	Normal formalinized spleen	1:40	++++	—	—	—	—	++++	—	—	—	—	—
		1:80	++++	—	—	—	—	++++	—	—	—	—	—
		1:160	+++	—	—	—	—	+++	—	—	—	—	—
		1:320	++	—	—	—	—	++	—	—	—	—	—
No. 97 to boiled tumor from patient P.	Normal formalinized spleen	1:40	++++	—	—	—	—	++++	—	—	—	—	—
		1:80	+++	—	—	—	—	+++	—	—	—	—	—
		1:160	±	—	—	—	—	+	—	—	—	—	—
		1:320	—	—	—	—	—	±	—	—	—	—	—
	Mixture of boiled tissues from organs of patient P	1:40	++++	—	—	—	—	++++	—	—	—	—	—
		1:80	+++	—	—	—	—	+++	—	—	—	—	—
		1:160	+	—	—	—	—	+	—	—	—	—	—
		1:320	±	—	—	—	—	—	—	—	—	—	—
	Boiled malignant tumor from patient P	1:40	—	—	—	—	—	—	—	—	—	—	—
		1:80	—	—	—	—	—	—	—	—	—	—	—
		1:160	—	—	—	—	—	—	—	—	—	—	—
		1:320	—	—	—	—	—	—	—	—	—	—	—

Conventional signs as in Table 1.

It is clear from Table 2 that the antitumor serum reacted only with antigens from untreated and boiled tumor tissue. Absorption of this serum with boiled spleen did not remove the specific antitumor antibodies from the serum. On the other hand, absorption of this serum with boiled tumor tissue completely deprived it of its ability to react with the tumor antigens.

It follows from these experiments that not only extracts but also tumor cells themselves, when heated to 100°, preserve their specific antigenic properties and, in contrast to spleen cells, can absorb specific antibodies from the serum.

We tested the integrity of the antigenic properties of the tumor after boiling by means of immunization experiments. The results of the serological study of one of the sera (No. 97) obtained by immunization of rabbits with a saline extract from the tumor in patient P, heated for 30 minutes to 100°, are shown in Table 3. For comparison in this experiment we also tested serum No. 211, prepared against untreated antigen from the same tumor. It will be seen from the results shown in Table 3 that antitumor serum No. 97, after removal therefrom of nonspecific antibodies (by absorption with normal formalinized spleen), was indistinguishable in its reaction from serum No. 211 and contained antibodies not only to the antigen from the boiled tumor tissue against which it was produced, but also led to fixation of complement in the presence of antigen from the untreated malignant tumor. Absorption of serum No. 97 with the tissues of patient P (tumor tissue and a mixture of organ tissues), heated to 100°, also revealed no serological differences between this serum and serum No. 211.

These experiments thus showed that antigen prepared from malignant tissue, after boiling for 30 minutes, was capable of causing the formation of specific antibodies when used to immunize animals.

Identical results showing the resistance of the specific antigens of malignant tumors to a high temperature were also obtained during the investigation of material from five other cases of cancer.

The results described in this communication concerning the difference in behavior between specific antigens of malignant tissues and the antigens of normal human organs to the action of a high temperature may be used for the purification and the separation of the tumor antigen in the course of its preparation.

SUMMARY

With the aid of immunological methods of investigation it was shown that the sensitivity of cancer tissues and healthy human tissues to the action of high temperature was different. Human cancers contain a specific antigenic component, resistant to boiling for the period of 30 minutes, in contradistinction to the organ specific antigens, destroyed in these conditions.

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EXPERIMENTAL DATA CONCERNING THE ISOPROPYL ESTER OF αN-NICOTINOYL-SARCOLYSIN

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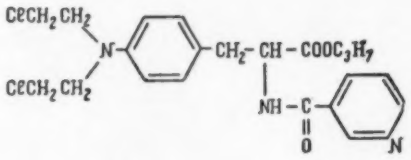
The idea on which was based the production of preparations of the type of the complex alkylating metabolites has been described previously [1, 2]. In some of the preparations which have been described and studied the complex carrier of the cytotoxic (chloroethylamine) group took the form of dipeptides. In the present preparation the carrier of the cytotoxic group is a compound of an amino acid (phenylalanine) and a vitamin (nicotinic acid).

The isopropyl ester of αN-nicotinoyl-sarcolysin has the following structure:



The compound was synthesized by E. N. Shkodinskaya, O. S. Vasina and A. Ya. Berlin in the chemical division of the Institute of Experimental and Clinical Oncology of the Academy of Medical Sciences of the USSR. In the present article we describe the results of an experimental investigation of this preparation.

EXPERIMENTAL METHOD

In order to study the antitimor action of the preparation different strains of transplantable tumors were used: in rats – spindle-cell sarcoma 45 and ascites hematoma (French strain); and in mice – Ehrlich's tumor (ascites and solid forms), carcinoma of the mammary gland (strains RMZh and MAP), carcinoma of the forestomach (OZh-5), sarcoma 298 and Crocker's sarcoma 180 (the last in ascites and solid forms).

In the case of the solid tumors administration of the preparations began when they reached a weight of 0.5-1.0 g in rats and 0.1-0.2 g in mice, and in the case of ascites tumors 24 hours after transplantation.

Since the preparation is insoluble in water it was administered to the animals in the form of a suspension in a 1-2% starch paste. The oral and intraperitoneal methods of administration were used. In solid tumors transplanted subcutaneously the preparation was given intraperitoneally or orally, and in ascites forms of the tumors it was always given intraperitoneally. In therapeutic experiments the preparation was given daily for 15-20 days.

The results of the experiments were evaluated as a percentage of inhibition and by rate of growth. In the experiments with ascites strains consideration was paid to the volume of ascites fluid, and the percentage of inhibition was calculated from the difference in the volume of ascites fluid in the experimental and control animals. The results were treated statistically by Student's method.

The study of the antitumor action of the preparation was carried out on 80 rats and 350 mice. In determining the toxicity of the preparation, it was injected into animals as a single dose. The results of these experiments were evaluated by the percentage of animals which died in the course of 30 days.

EXPERIMENTAL RESULTS

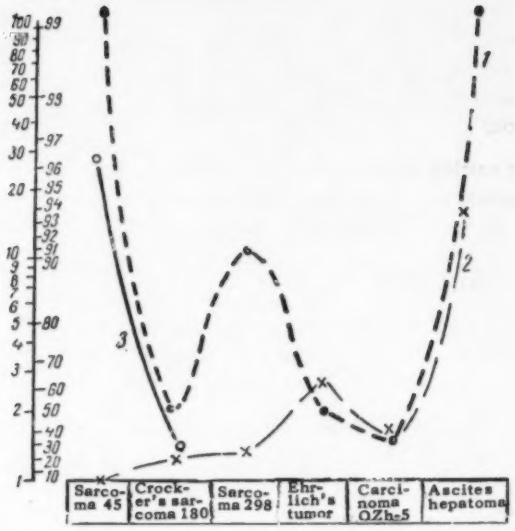
The toxicity of the preparation was first studied. The experiments showed that it possesses low toxicity; for instance, a dose of 1000 mg/kg, when injected once intraperitoneally, did not cause the death of rats and mice. Doses of 175-300 mg/kg, when administered daily for 15 days, were well tolerated by the animals.

The effect of the preparation on the blood was studied in a therapeutic experiment with Ehrlich's tumor. It was found that the white cell count in the peripheral blood remained normal when the preparation was given in a dose of 200 mg/kg daily for 15-20 days. A decrease in the weight of the spleen by one-third or one-half was, however, observed under these conditions. The results relating to the antitumor action of the preparation are given in the table.

The Action of the Isopropyl Ester of αN-Nicotinoyl-Sarcolysin

Strain of tumor	Dose (in mg/kg)	Mode of administration	Number of animals	Mean weight of tumor (in g)		Volume of ascites fluid (in ml)		Inhibition (in %)	Criterion of significance
				control	expt.	control	expt.		
Ascites hepatoma	200	Intraperitoneally	30	–	–	37	2.5	93	1
Mammary gland carcinoma (strain RMZh)	300	Orally	30	0.84	0.29	–	–	65.5	0.998
	200	Intraperitoneally	20	0.85	0.12	–	–	86	1
Strain MAP	300	Orally	40	2.9	2	–	–	31	0.9
Ehrlich's tumor (solid form)	200	Intraperitoneally	60	1.4	0.55	–	–	60 (44-72)	0.999
	300	Orally	30	1.2	0.65	–	–	46	0.999
Ehrlich's tumor (ascites form)	175	Intraperitoneally	20	–	–	2.8	1.6	43	0.95
Carcinoma of forestomach OZh-5	175	"	20	2.7	1.8	–	–	33	0.95
Sarcoma 298	200	Orally	48	2.7	2	–	–	25.5	
Crocker's sarcoma 180	175	Intraperitoneally	74	0.5	0.4	–	–	20	0.45
	300	Orally		0.6	0.57	–	–	0	
Ascites form	175	Intraperitoneally	30	–	–	2.3	1.7	26	
Sarcoma 45	200	"	50	37.5	40.7	–	–	0	

It will be seen from the table that the ascites hepatoma of rats was most sensitive to the preparation. For instance, in a dose of 200 mg/kg, when injected intraperitoneally for 13 days, the preparation inhibited growth of the ascites hepatoma of rats by 93%. In most of the treated animals traces of ascites fluid remained at the end of the experiment, but in control animals the volume of ascites reached 37 ml.



Spectra of antitumor action of the isopropyl ester of αN-nicotinoyl-sarcolysin and closely related compounds. 1) Sarcolysin; 2) isopropyl ester of αN-nicotinoyl-sarcolysin; 3) isopropyl ester of sarcolysin. Along the axis of abscissas are shown the forms of tumor; along the axis of ordinates (graduated in a logarithmic scale) are the indices of inhibition for each strain (on the left) and the corresponding percentages of inhibition (on the right).

The preparation also inhibited growth of the mammary gland tumor (strain RMZh) by 65-86% and of Ehrlich's tumor by 44-72%. The action of the preparation on the ascites and solid forms of Ehrlich's tumor was practically identical. The preparation had no action on sarcoma 45 of rats or on Crocker's sarcoma and sarcoma 298 in mice.

It was thus established that the preparation possesses some antitumor activity. Epithelial tumors, namely the ascites hepatoma of rats and carcinoma of the mammary gland (strain RMZh), were most sensitive to the preparation.

When the results of this investigation are compared with the data obtained in our laboratory by Sof'ina [3, 4] and Trusheikin [5] from studies of the action of sarcolysin and the isopropyl ester of sarcolysin, certain differences are found in the spectrum of action of these preparations. We show in the figure the spectrum of the antitumor action of these preparations (drawn from Larionov's scheme).

It can be seen from the figure that sarcolysin, and also the isopropyl ester of sarcolysin, have a strong inhibitory action on sarcoma 45 and sarcoma 298, whereas the isopropyl ester of αN-nicotinoyl-sarcolysin has no action whatsoever on these tumors. Both sarcolysin and the isopropyl ester of αN-nicotinoyl-sarcolysin, however, had equal effects on ascites hepatoma of rats, Ehrlich's tumor and carcinoma of the forestomach.

The analysis of our findings confirms Larionov's view [1] that, by changing the carrier of the functional group, preparations having a different spectrum of action can be created.

SUMMARY

The author studied the antiblastic effect of isopropyl ester of αN-nicotinoyl-sarcolysin on various strains of transplantable tumors. Epithelial tumors, namely rat ascitic hepatoma (French strain) and carcinoma of the mammary gland (CMG strain), proved to be the most sensitive to the preparation. The latter exerted no effect on rat sarcoma 45 or on Crocker's sarcoma 298 in mice. The strength and the range of the antiblastic effect of this preparation differed from that in similar compounds – sarcolysin and isopropyl ester of sarcolysin.

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ONCOLOGICAL CHARACTERISTICS OF A NEW STRAIN OF RAT LYMPHOSARCOMA

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As the number of chemotherapeutic drugs for use against cancer and tested in animals increases, and as biochemical and experimental oncological research is intensified, interest in tumors transplantable into animals increases daily.

The survey made by Pogosyants [2], devoted to the description of tumor strains, gives an extensive list of strains available in Soviet laboratories. Despite the great variety of transplantable tumors in this list, it does not include strains of lymphosarcoma transplantable into rats. Such strains are nowadays widely used in the practice of oncological laboratories abroad [3-5]. We therefore considered it expedient to describe a new strain of lymphosarcoma which we have obtained, which is transplantable into rats.

The tumor was found in a 3½ month old female rat, kept from birth on a diet containing 3,3'-dichlorobenzidine, a substance whose high carcinogenic activity was demonstrated by us in 1958 [1]. The parents of this rat had also received this substance from birth in the same dose (20 mg). Consequently, the animals had been under the influence of a carcinogen throughout their lives.

In the rat which we are describing, a greyish-white neoplasm measuring 6 × 4.5 × 4.5 cm was found in the abdominal cavity above the duodenum and transverse colon, firmly adherent to the mesentery and to its root (Fig. 1). The tumor was invading the retroperitoneal cellular tissue and infiltrating the tissue of the kidneys. Solitary tumor nodules were also observed in the mesentery. The peripheral lymphatic glands, liver and spleen were not enlarged; the bone marrow was pale crimson in color.

Miscoscopic examination of the tumor (Fig. 2) showed that it consists of large and small lymphoid cells, round or irregular in shape, with large round or bean-shaped nuclei usually displacing nearly all the basophilic cytoplasm of the cell, lying in close contact with each other. The moderately hyperchromic nuclei contained one or two nucleoli and delicate chromatin granules, evenly distributed throughout the nucleus. Very many figures of mitotic division of the nuclei were present. Cells of this type were found in large numbers inside the lumen of the vessels in various organs. The tumor infiltrated the kidneys and invaded the pancreas. Areas of perivascular infiltration were found in the lungs. Only in isolated areas of the spleen were small collections of lymphoid cells seen. In the perivascular sheaths in the liver very small foci of lymphoid hemopoiesis were encountered here and there. No lymphoid metaplasia of the bone marrow was observed. The peroxidase reaction gave negative results. From the morphological picture of the changes we were inclined to consider that this lesion was a lymphosarcoma, probably arising from the lymphatic glands at the root of the mesentery or the retroperitoneal cellular tissue, with generalization of the process to involve several organs.

Fig. 1. The rat with the tumor in the abdominal cavity. The neoplasm from which the strain originated.

A homogenate of the tissue of the principal nodule was inoculated in a dose of 0.5 cm^3 into the subcutaneous cellular tissue of eight rats of both sexes, weighing 70-120 g. Five days later at the site of inoculation in all the animals the skin was elevated by infiltration, which in three rats weighing 70 g was subsequently absorbed. In five rats the tumor continued to grow and reached a large size.

For the subsequent transplantations we used mainly male rats weighing 90-110 g. A suspension of tumor cells in a dose of 0.5 cm^3 was injected through a wide needle into the subcutaneous cellular tissue of the dorsum of the animal. On the 5th-7th day at the site of transplantation small nodules were found, which increased in size fairly rapidly, invaded the skin and formed ulcers. The tumor was immobile and infiltrated widely into the subcutaneous cellular tissue. In the first 29 generations the tumor had no well defined borders, but it spread far beyond the limits of the site of transplantation, and apparently splitting the subcutaneous cellular tissue into two layers, it covered the entire rat in the form of a muff (Fig. 3), penetrating the deep and the retroperitoneal cellular tissue. Later the character of its growth altered: at the site of transplantation a nodule developed, increased in size progressively and showed no marked tendency towards widespread invasion of the surrounding tissue, although in some generations invasion of the retroperitoneal cellular tissues was observed. In the first generations the tumor had a doughy consistency, and in later generations it was firmer; the tumor was greyish-white in color, and an opaque fluid of a white color flowed from its cut surface. In the center of the neoplasm small areas of necrosis were observed.

The tumors attained the size of 9 × 6 × 4 cm. The duration of life of the rats with tumors varied from 12 to 95 days. Most animals, however, perished on the 30th-40th day of the experiment. Transplantation was carried out on the average after 13-16 days. The proportion of successful transplantations varied from 75 to 100% (Table 1).

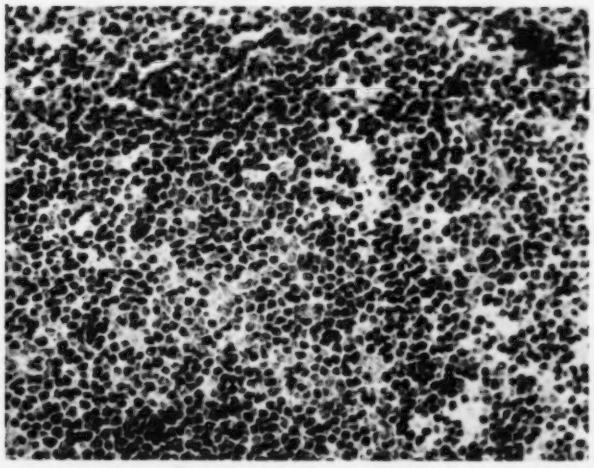

Fig. 2. Microscopic structure of the lymphosarcoma. Magnification 400 ×.

TABLE 1

Results of Transplantation of the New Strain of Rat Lymphosarcoma

Generation	Number of animals included in experiment	with successfully transplanted tumors	with tumors undergoing spontaneous regression	with unsuccessfully transplanted tumors	Successful transplants (%)
1	8	5	3	—	62.5
2	5	4	1	—	80
3	5	3	2	—	60
4	5	4	1	—	80
5	4	4	—	—	100
6	4	4	—	—	100
7	5	5	—	—	100
8	5	4	1	—	80
9	5	5	—	—	100
10	5	5	—	—	100
11	5	5	—	—	100
12	4	3	1	—	75
13	4	4	—	—	100
14	4	4	—	—	100
15	4	4	—	—	100
16	5	5	—	—	100
17	3	3	—	—	100
18	4	4	—	—	100
19	4	4	—	—	100
20	5	5	—	—	100
21	4	4	—	—	100
22	4	4	—	—	100
23	4	4	—	—	100
24	5	5	—	—	100
25	5	5	—	—	100
26	6	6	—	—	100
27	5	5	—	—	100
28	4	4	—	—	100
29	5	5	—	—	100
30	5	4	—	1	80
31	8	8	—	—	100
32	5	5	—	—	100
33	5	5	—	—	100
34	4	4	—	—	100
35	4	4	—	—	100
36	4	4	—	—	100
37	4	3	1	—	75
38	4	4	—	—	100
39	4	3	1	—	75
40	5	5	—	—	100
41	5	5	—	—	100
42	5	5	—	—	100
43	4	4	—	—	100
44	5	4	1	—	80
45	5	5	—	—	100
Total	211	198	12	1	93.8

It must be emphasized that we observed no significant changes in the morphological picture of the tumo. in the course of 45 generations (until November 10, 1959). The nodules consisted of the same young lymphoid cells which were described in the tumor in the original rat.

The animals which died or were sacrificed at late stages of the experiment merely showed signs of ex- exhaustion. The liver, spleen and lymphatic glands were not enlarged. Here and there in the liver were seen small collections of young lymphoid cells in the perivascular sheaths; in the spleen were seen foci of increased proliferation of reticulum cells and of infiltration with lymphoid cells.

TABLE 2

Blood Count (mean of 8 counts) in Rats with Transplanted Lymphosarcoma (new strain)

Rat	Erythroblasts	Leucocytes (thousands/$/mm^3$)	Neutrophils (in %)			Eosinophils	Lymphocytes	Monocytes
			juvenile	stab cells	segmented			
						%		
Control	–	12 ± 1,3	–	7	12	1	75	5
Experimental	6	13 ± 3	2	5	7	4	72	4

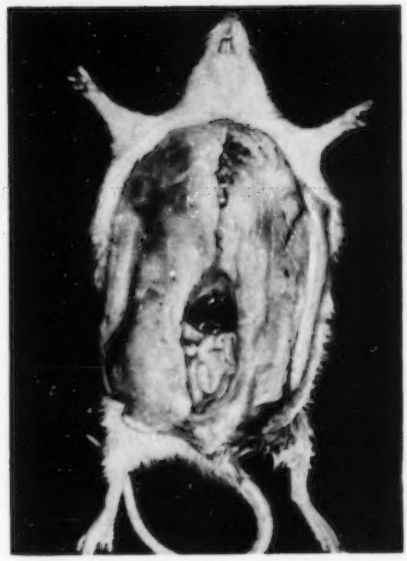

Fig. 3. A rat on the 49th day after subcutaneous transplantation (5th generation). The tumor is infiltrating the subcutaneous cellular tissue.

On comparing the blood and marrow films of healthy and experimental animals we were unable to detect any appreciable increase in the number of lymphoid cells in the experiment. As is clear from Table 2, only a slight shift in the leukocyte formula to the left and an increase in the number of erythroblasts were observed.

An attempt at transplantation using blood and homogenate of liver and spleen (taken from rats with a subcutaneous tumor), carried out three times on 21 rats, on two occasions did not give positive results, although in one case two of five rats developed lymphosarcoma at the site of injection of the homogenate.

A new transplantable strain of rat lymphosarcoma has been obtained and observed in 45 generations. The findings described indicate that this strain of tumor possesses certain distinctive features, including the fact that in some rats, in addition to the development of a neoplasm in the subcutaneous cellular tissue, generalization of the process may occur.

SUMMARY

The author describes a new strain of rat lymphosarcoma. The initial tumor was detected in the abdominal cavity of a female rat which was fed 3,3'-dichlorobenzidine from birth. When transplanted the tumor took in 75-100% of the cases. Generalization of the process occurred occasionally in subcutaneous transplantation. In a number of cases (averaging 5.7%) "spontaneous" regression was observed.

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NORMAL ANTIBODIES DURING REGENERATION IN INVERTEBRATE ANIMALS

COMMUNICATION 1. NORMAL ANTIBODIES AGAINST REGENERATING TISSUE IN THE RAY OF Asterias rubens

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Experimental researches in vertebrates [3-5] have shown that the processes of regeneration developing after disturbance of the integrity of the tissues (fractures, blood loss, burns, freezing, partial ablation of an organ) are accompanied by the production of specific autoantibodies. It might be expected that a similar process would also be observed in invertebrate animals. If such a pattern were established it would enable progress to be made toward the explanation of the nature of the primary immunological response and of the role of normal antibodies in morphogenetic processes [1].

In order to investigate the problem of the possible detection of specific autoantibodies to regenerating tissue in invertebrate animals we carried out an experiment on the starfish Asterias rubens, whose marked regenerative powers are well known.

EXPERIMENTAL METHOD

For the experiment we used 30 uninjured starfish and 30 starfish with a regenerating ray. In control experiments we used the polychaete worm Arenicola marina (20 specimens).

In order to verify that normal antibodies were present in the celomic fluid of the above-mentioned invertebrate animals, the ring precipitation test was carried out. As "serum" we used a lyzate of the cells of intact starfish and of others from which one ray had been removed, prepared in distilled water. The lyzate was prepared from the residue of the celomic fluid of the starfish after centrifugation at 2000 rpm for 5 minutes. The supernatant fluid was poured off into another test tube, and distilled water in the same volume was added to the residue. After careful mixing for 30 minutes, chemically pure sodium chloride was added to the solution to make the salt concentration of the solution equal to that of sea water (0.25 g NaCl to 100 ml sea water). The lyzate prepared in this way and also the supernatant fluid were used in the experiment in undiluted form.

Tissue extracts (antigens) for the ring precipitation test were prepared in filtered sea water in a concentration of 1:10. For the experiment we prepared antigens from the tissues of the uninjured starfish, of the regenerating ray of the starfish, and from the tissues of the annelid worm. All antigens were used in a dilution of 1:100. Into the control tubes filtered sea water was added instead of one of the reagents listed above.

The results of the ring precipitation test were read after the tubes had been kept for 5-10 minutes at room temperature. Each experiment was repeated 2-3 times. The degree of the reaction was expressed according to the following scale: – no ring; ± indistinct ring; + fine ring; ++ clear, dense ring.

During the performance of the ring precipitation test certain peculiarities in its course were observed. In all the experiments with untreated tissue fluids the reactions were positive only if these fluids were prepared not later than 18 hours before carrying out the test. In the experiments with tissue fluids prepared 12, 8, 6 or fewer hours before the test, there were no positive reactions. On the other hand, the lyzate of the cells from the celomic fluid of the starfish, used as serum, reacted even when it was prepared ex tempore. When carrying out the experiments we therefore used lyzate prepared ex tempore and tissue extracts prepared not later than 18 hours before the test.

EXPERIMENTAL RESULTS

The results of the experiments are shown in the table.

Ring Precipitation Test Between "Sera" of an Uninjured Starfish and a Starfish with a Regenerating Ray and Tissue Extracts from an Uninjured Starfish, a Starfish with a Regenerating Ray and the Annelid Worm Arenicola marina

Undiluted "serum" / Antigen (1:100)	"Serum" of uninjured starfish		"Serum" of starfish with regenerating ray		Sea water
	celomic fluid	lyzate of cells	celomic fluid	lyzate of cells	
Uninjured Asterias rubens	±	++	–	+	–
Asterias rubens with regenerating ray	–	–	±	++	–
Arenicola marina	–	++	–	++	–
Sea water	–	–	–	–	

It can be seen from the table that the celomic fluid of the uninjured starfish reacted with the tissue extract of the same starfish to a degree ± , but did not react with the tissue extract of the starfish with the regenerating ray.

The lyzate of the cells from the celomic fluid of the uninjured starfish reacted with the tissue extract of the same starfish, giving a clear, dense ring evaluated as ++, but did not react with the tissue extract of the starfish with the regenerating ray. The celomic fluid of the starfish with the regenerating ray reacted with the tissue extract of the same starfish (±) but did not react with the tissue extract of the uninjured starfish. The lyzate of the cells from the celomic fluid of the starfish with the regenerating ray reacted with the tissue extract of the uninjured starfish, forming a fine ring (+), and with the tissue extract of the starfish with a regenerating ray, forming a clear, dense ring (++). The celomic fluids of the starfish, both uninjured and with a regenerating ray, did not react with the tissue extract of the annelid worm. The lyzate of the uninjured starfish and of the starfish with the regenerating ray reacted distinctly with the tissue extract of the annelid worm, giving a dense ring (++).

Thus the celomic fluids of both the uninjured starfish and the starfish with the regenerating ray reacted weakly with the corresponding tissue extracts and did not react at all with the other extracts. This may evidently be explained by the fact that the greater part of the normal antibodies is contained in the cells and that only an insignificant part of them is present in the celomic fluid. Increased destruction of the cells leads to the release of these antibodies into the solution. This is confirmed by the fact that the lyzate of the uninjured starfish reacted only with the tissue extracts from its own tissues (or the tissues of another uninjured starfish) and with the tissue extracts of the annelid worm.

The lyzate of the starfish with the regenerating ray reacted with the tissue extracts of its own regenerating tissue (or the regenerating tissue of another starfish), and also with the tissue extracts of the uninjured starfish and of the annelid worm. In other words, the cells present in the celomic fluid of the starfish with the regenerating ray contained normal antibodies against its own regenerating tissue or the regenerating tissue of another starfish, but these antibodies could not be detected in the uninjured starfish.

The results obtained thus suggest that the process of regeneration in invertebrate animals is accompanied by the appearance of autoantibodies against the regenerating tissue in the cells of the celomic fluid. However, cross tests with these autoantibodies and tissue extracts of other species of invertebrates do not yet permit final conclusions to be drawn regarding their specificity. Further experiments with adsorption of nonspecific antibodies are required.

SUMMARY

The possibility of detection of specific autoantibodies to the regenerating tissue was studied. Experiments were carried out on Asterias rubens with employment of the ring precipitation tests. Cavital fluids with their cellular elements of intact and regenerating Asterias rubens served as "sera" and tissue extracts of their tissues as "antigens." The results of experiments have demonstrated that in the cavital fluid of the starfish with regenerating ray there are antibodies to their own regenerating tissues or those of other Asterias rubens. No such antibodies were revealed in the cavital fluids of intact starfish.

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THE EFFECT OF OVERSTRAIN OF DIFFERENTIAL INHIBITION ON THE REGENERATION OF SKELETAL MUSCLE IN WHITE RATS

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The problem of the influence of the functional state of the cerebral cortex on the complex morphophysiological processes taking place in repair is one of great theoretical and practical interest. We have previously [8, 9] studied the question of the influence of overstrain of the cortical process of excitation on the traumatic regeneration of skeletal muscle.

In the present communication we describe the results of experiments to show the influence of overstrain of the process of differential inhibition on the regeneration of skeletal muscle.

EXPERIMENTAL METHOD

For the experiments we used 48 white rats weighing 300-400 g (20 control and 28 experimental animals). As regards the type of nervous system of the experimental rats, 13 were excitable, and 15 were inhibited. A system of two positive and two inhibitory stimuli was produced in these rats. A continuous light from a 10 v electric lamp and a bell of medium strength were used as positive conditioned stimuli, and a winking light and buzzer were the inhibitory stimuli. After the production of this system the next step was to create overstrain of the process of inhibition, which was achieved by prolonging the time of action of the differential stimuli from 20 seconds to 2-3 minutes and by increasing their number in the course of one experimental day from two to 5-7. Ten days after the start of the experiments with overstrain of inhibition, a muscle in both hindlimbs of the rats was injured. The anterior tibial muscle was divided with scissors for two-thirds of its width in its middle part. The operation was carried out in strictly aseptic conditions under ether anesthesia. The process of healing of the muscle wound was studied by means of serial sections through the wound (thickness of the sections 6-8 μ). The material was fixed in Helly's mixture 12, 24 and 48 hours and 5, 8, 12, 16, 20 and 30 days after the wounding of the muscle. Paraffin wax sections were stained with suitable histological stains. The experiments with overstrain of inhibition continued after the operation, until the time that the material was taken for histological analysis. The process of regeneration in the muscle thus took place while the cortical activity of the animals was chronically disturbed.

EXPERIMENTAL RESULTS

In the group of excitable rats overstrain of differential inhibition after the first day of the experiment led to intensification of the motor food reaction. After a phase of general excitation, lasting about two weeks, there developed a short period (3-5 days) of relative localization of the processes of excitation and inhibition. Subsequently a sharp increase in excitation and a weakening of differential inhibition were observed, in which the food taking reaction was strengthened (the rats drew the feeding bowl towards them, and gnawed the floor and

the walls of the chamber). The signs of nervous difficulty increased. The number of times that disinhibition of differentiation took place increased, and during these times and in the intervals between them the rats performed training movements, which terminated by the fatigue of motor excitation. In the course of ten days the rats lost 20-30 g in weight. The state of sharply increased excitation then gave way to the ever increasing development of diffuse inhibition, in which 2-3 positive stimuli acting on different analyzers weakened it only for a short time.

The second group of rats (inhibited) possessed a weaker nervous system. During the introduction of differentiation the conditioned food reflexes in these animals disappeared, and were restored only one week later. The reaction of the cerebral cortex to the overstrain of differential inhibition also took place in these rats in the form of two principal phases: a state of strong food excitation and a state of food inhibition. In contrast to the excitable rats the first phase was shorter and the second deeper.

A description of the process of healing of the muscle defect in the control rats was given in our previous communication [9], and we shall not concern ourselves with it here.

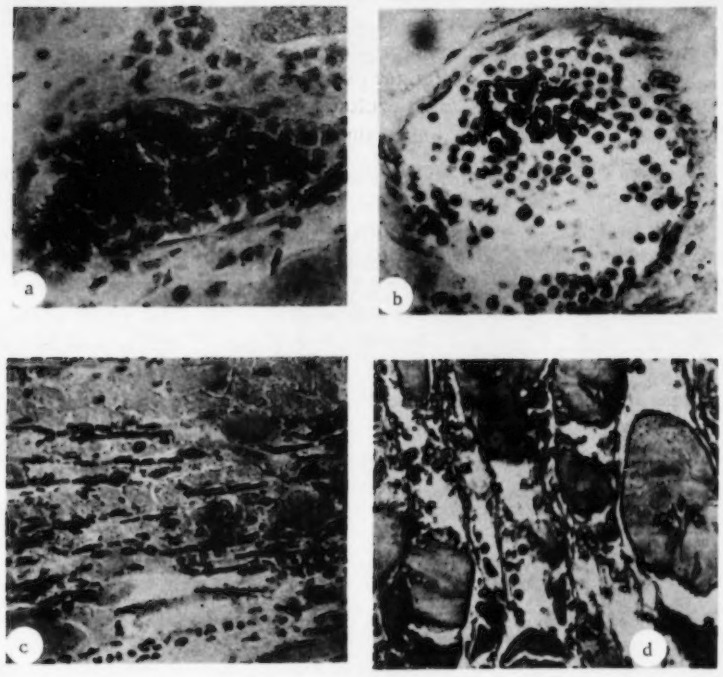
In the rats with an excitable type of nervous system the disturbance of cortical activity affected the course of healing of the muscle wound, especially in its early stages. The inflammatory reaction was accompanied by a very intensive leukocytic infiltration of the wound. Resolution of the fibrin threads and resorption of the necrotic cells and tissues took place more energetically than in the control rats. At the end of the first day the wound was completely cleansed of large fragments of destroyed muscle, which indicated the increased activity of the cells taking part in the cleansing of the wound. Judging by the considerable accumulation of fibroblastic cells in the focus of injury and by the large number of mitotic figures observed in many fields of vision in the sections, there was a more active connective tissue reaction in the experimental than in the control rats. The regenerative reaction of the muscle tissue on the 2nd-5th day of healing was also more intensive than in the control rats. The myosymplasts gave off more side branches, which were distinguished by their greater caliber and spread. The distal ends of many of these branches, however, showed signs of vacuolation and disintegration. On the eighth day the connective tissue showed proliferation of its fibers, which was especially marked in the central area of the wound, where it caused the death of many of the young muscle fibers (see figure).

Judging by the intensity of their staining by Mallory's method and by their density, the degree of development of the collagen bundles corresponded to the 12th day of regeneration in the control rats. Until the 16th day intensive development of collagen bundles and delay of the growth of young muscle fibers were observed. On the 20th day the regenerating wound presented a very complex histological picture. Collagen bundles and young muscle fibers ran about in different directions and were interwoven with each other. Among the young muscle fibers there were some which were fully differentiated and contained well defined structures, whereas others had no cross striation and contained collections of round nuclei; fibers were seen which were strongly contracting, fibers showing signs of disintegration and, among them, slender myosymplasts with a well marked basophilia. At the 30th day the defect was filled with a complex binding of regenerated muscle fibers and connective tissue septa. Many muscle fibers were deformed and had ill defined borders, and were distinguished by their degree of differentiation and their caliber.

The main feature of the process of regeneration in the excitable rats during overstrain of differential inhibition was thus an increased connective tissue reaction, and this was already observable in the early stages of healing of the muscle wound.

In the inhibited rats the course of the process of inflammation revealed a feeble leukocytic infiltration of the wound, due to delay in the dispatch of leukocytes from the blood vessels. Twelve hours after injury in sections through blood vessels collections of leukocytes could be seen close to the inner wall, and outside the walls of the vessels only isolated leukocytes were encountered. In the control rats, on the other hand, collections of leukocytes were found in the outer walls of the vessels, indicating that they had passed out of the blood stream. After 24 hours the wound in the inhibited rats contained much tissue fluid and large muscle fragments, with little or no sign of phagocytic activity. The newly formed blood vessels also were poorly developed.

On the fifth day the injured muscle fibers became intensively basophilic, and partially lost their structure. The cross striation disappeared, their fibrillary structure was preserved at the periphery of the fiber, and chains of nuclei showing amitotic division occupied a central axial position. On the eighth day these fibers underwent complete dedifferentiation and were converted into long myosymplastic bands. The regeneration of the connective tissue was less marked than in the control rats and much less than in the excitable rats. Mitoses were rarely



Inflammatory process in the muscle in the control and experimental (inhibited) rats. a)Control rat 12 hours after operation. Transverse section through a blood vessel. In the outer walls of the vessel groups of leukocytes are seen. b)The same, after 24 hours. In the wound may be seen highly fragmented and disintegrating muscle, groups of phagocytes and newly formed capillaries. Stained with azure II–eosin. Magnification: objective 40 ×, ocular 5 ×. c) Overstrain of differential inhibition. Inhibited rat, 12 hours after operation. Transverse section through a blood vessel. In the vessel may be seen leukocytes, mainly situated near the inner wall, only a few leukocytes being present in the outer walls of the vessel. d) Overstrain of differential inhibition after 24 hours. In the wound may be seen large fragments of disintegrating muscle and much tissue fluid. A few feebly developed newly formed blood vessels. Small groups of phagocytes. Stained with azure II eosin. Magnification: objective 40 ×, ocular 5 ×.

seen in the fibroblasts (5th day) and on the eighth day fine collagen bundles were formed. Until the 20th day collagen formation was feeble, which provided favorable conditions for the regeneration of muscle fibers. In fact numerous myosymplasts appeared in the regenerating muscle tissue, and the muscle tubes contained an enormous number of nuclei, which indicated an increase in their proliferative activity. At the same time the differentiated structures in the muscle tubes were ill defined. On the 30th day the quantity of connective tissue in the wound increased appreciably, and the collagen bundles proliferated and grew thicker (staining an intense blue with Mallory's stain), while the newly formed muscle fibers lagged behind those in the control rats in their degree of differentiation (feeble cross striation, many nuclei) but their diameter did not differ from that in the controls. The impression was created that, besides a weakening of the processes of collagen formation, which was observed until the 20th day, the coordination between the growth of the muscle elements and their differentiation was disturbed.

The connective tissue reaction is of great importance to the regeneration of muscle tissue. Connective tissue is an essential supporting component of the regenerating muscle, accompanying the growth of the young muscle fibers [16]. We have shown [9] that in the course of regeneration the relationship between muscle and

connective tissues may be changed by influencing cortical excitation. In the present investigation a similar result was obtained by influencing the process of internal (differential) inhibition. In both cases differences clearly emerge in the healing of the muscle defect in rats with different types of nervous system. During overstrain of the process of inhibition, regeneration of muscle took place in association with alternate states of increased and diminished motor food excitation in the cerebral cortex. In the phase of the maximum level of motor food excitation (until the 16th-20th day) increased regenerative activity of the connective tissue was observed, which restricted regeneration of the muscle tissue. After the 20th day healing took place in association with inhibited cortical activity. At this period a reduction in the amount of connective tissue in the regenerating muscle was observed by comparison with the 16th day, but it was still greater than in the controls. In the inhibited rats the early period of regeneration was accompanied by a weakened connective tissue reaction, and at this time a state of inhibition dominated the cortical activity of the rats. In the phase of increased motor food excitation (12th-20th days) the collagen fibers in the regenerating muscle as before were more feebly developed than in the control rats, and only on the 30th day was there an appreciable increase in the quantity of connective tissue. These comparisons reveal how extraordinarily complex are the relationships between the cortical and regenerative processes. The changes in the reaction of the connective and muscle tissues are the result of disturbance of the trophic influences of the nervous system, which are connected with the active working of the cortical cells. In reaching these conclusions we have taken into consideration the facts discovered by several workers during disturbances of the activity of the cerebral cortex [6, 13, 16, 18, 19, 21, 22].

SUMMARY

Overstraining of differential inhibition renders significant effect upon the process of traumatic regeneration in the skeletal muscle. In rats with the excitatory type of the nervous system the inflammatory reaction is very vivid. Healing of the muscular wound is associated with intensive growth and connective tissue differentiation, inhibiting growth of the young muscle fibers and provoking atrophic changes in them. In rats with the inhibitory type of the nervous system the process of wound cleaning is delayed. The collagen formation is reduced up to the 20th day, and not much connective tissue is seen in the wound. The muscular tissue regeneration in this case is characterized by an intensive formation of myosymplasts and their retarded differentiation.

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COMPARATIVE CYTOCHEMICAL STUDY OF SUCCINIC DEHYDROGENASE AND ALKALINE PHOSPHATASE ACTIVITY IN PRIMARILY EXPLANTED AND TRANSPLANTABLE TISSUE CULTURE CELLS

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It was previously shown that during the first three days of culturing of first generation renal cells alkaline phosphatase [2] and succinic dehydrogenase [3] activity in these cells markedly decreases. On the other hand, NEr-2 strain cells exhibited a considerable succinic dehydrogenase activity at all times. It was noted that endogenous dehydrogenase activity was relatively high in the first generation renal cells and low in NEr-2 strain cells [3]. However, it seemed advisable to check this previously received data on other tissue cultures, including primarily explanted and transplantable cells of the same origin. The literature pertinent to the question is cited [2, 3].

EXPERIMENTAL METHOD

The cells studied included NEr-2 strain, heart cells of a monkey [Macacca cynomolgus (SOZ)], human amnionic epithelial cells,* first generation amnionic cells and second generation Macacca rhesus renal cells. The methods of culturing cells and determining enzymes were described previously [2, 3]. The cells were removed from the glass surface with 0.03% versene and, in some experiments with SOZ cells, with 0.25% trypsin. The enzymatic activity was studied during 9 to 10 days of culturing, in the cell suspension immediately following treatment with trypsin (or versene) and in frozen sections of amnionic membranes. Each tissue culture was subjected to three series of experiments. Trypsin treatments of SOZ cells were repeated twice.

EXPERIMENTAL RESULTS

Alkaline phosphatase. Alkaline phosphatase activity was relatively low in the freshly frozen epithelial cells of the amniotic membrane and in the cell suspension immediately following treatment with trypsin. Cobalt sulfide precipitate, according to which the distribution and presence of enzyme was judged, was almost entirely absent during the first three days of culturing. In the ensuing days the amount of precipitate increased. In general the alkaline phosphatase activity level in the cells of amniotic membrane, cell suspension, and culture was relatively low.

The incubation time for determining succinic dehydrogenase in the tissue culture cells was shortened from 2 hours to 1 hour and even to 30 minutes as compared to the previously reported technique [3]. This was done in order to obtain better localization of enzyme activity in the cells of transplantable strains.

* The strain of these cells was obtained at the Moscow Research Institute for Antipoliomyelitis Preparations by T. G. Orlova and was kindly provided by her for our study. Treatment with trypsin and culturing the first generation amniotic cells were also performed by T. G. Orlova, for which we are extremely grateful to her.

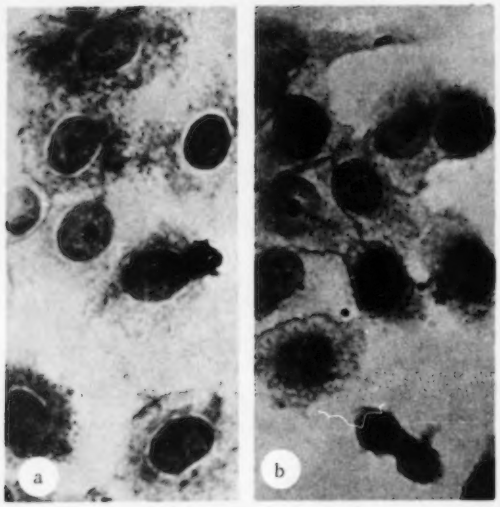

Fig. 1. Alkaline phosphatase activity in transplantable cells. First day of culturing. a) Amniotic cells treated with versene; b) SOZ cells treated with trypsin. Photomicrographs × 500.

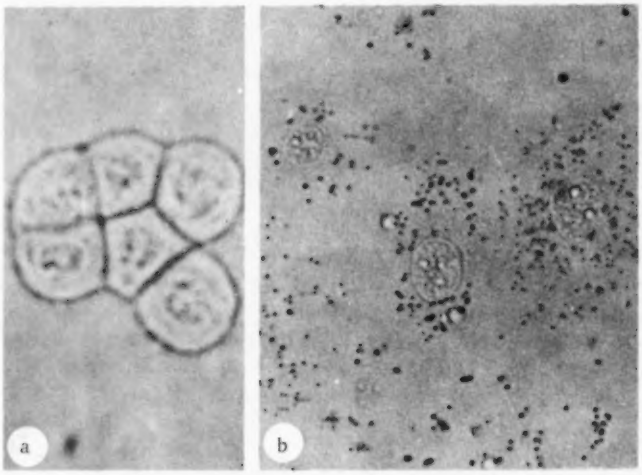

Fig. 2. Succinic dehydrogenase activity in primarily explanted amniotic cells. a) First day of culturing; b) seventh day of culturing. Photomicrographs × 500.

In studying renal tissue subculture it was found that cells in suspension as well as cells in culture had a very low alkaline phosphatase activity level. It was noted that the second generation of renal tissue culture was composed of "spindle" and "flat" cells, while the first generation of renal tissue culture cells showed enzyme-containing "nest" cells [2]. However, it should be noted that it was possible to find individual cells with high alkaline phosphatase activity in a few preparations.

In distinction from primarily explained cultures, the cells of all the transplantable strains examined posessed high alkaline phosphatase activity. The intensity of reaction during the first few days of culturing

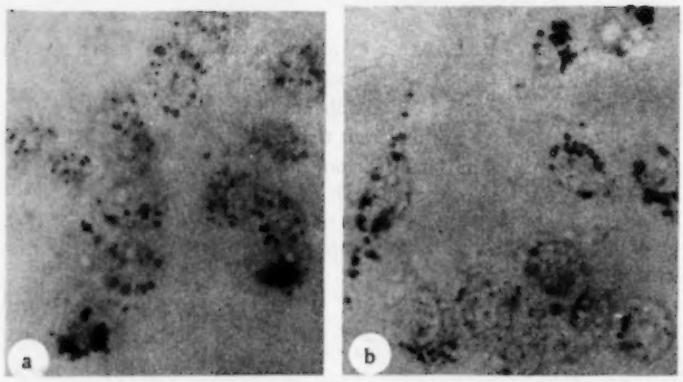

Fig. 3. Succinic dehydrogenase activity in transplantable cells. a) Amniotic cells, first day of culturing, treated with versene; b) amniotic cells, 6th day of culturing. Photomicrographs x 500.

was only slightly lower than in the cell suspension immediately following treatment with versene (Fig. 1a). The treatment with trypsin solution, as it was performed in two series with SOZ cells, did not lead to a decrease in enzyme activity (Fig. 1b). It was of interest to note that in transplantable cells of human amniotic epithelium the intensity of reaction was just as high as it was in other transplantable cell strains, despite the fact that enzymatic activity was very low in primarily explanted amniotic cells. It must be noted that while the general content of cobalt sulfide precipitate was high in the transplantable cells, there also were a few cells with relatively low intensity of enzymatic reaction.

Succinic dehydrogenase. Moderate deposits of diformazan granules were found in cells of unfixed frozen sections of human amnionic membrane. Cells in suspension, immediately following treatment with trypsin, likewise contained a moderate precipitate. The intensity of reaction in some cells was minimal. A striking decrease in enzymatic activity occurred during the first 2 to 3 days of culturing (Fig. 2a). Only on the 4th day did some cells show diformazan granules. The intensity of reaction increased up to 6 to 7 days (Fig. 2b), stabilized at this level and began to decrease from the 10th to 11th days. The endogenous activity of dehydrogenases in the cells of the 3 to 7 day culture was well expressed, and its level was only slightly lower than that of succinic dehydrogenase activity.

The dynamics of enzymatic activity changes were uniform in all transplantable cell strains studied. The cell suspensions from these cultures were extremely rich in needle-like crystals of diformazan. The reaction was absent only in isolated cells and could be explained by nonviability of these cells. Likewise endogenous dehydrogenase activity level was relatively high in cells from suspensions. During the 1st day of culturing, despite some decrease, the activity of succinic dehydrogenase was still high (Fig. 3a). By the 2nd day the intensity reached a very high level, and maintained it up to the 7th and 8th days.

Diformazan precipitate which was formed in transplantable amnionic cells was more delicate than in other transplantable cultures. This could be probably explained by the "young" age of the cell strain, which was started in March, 1959. Endogenous dehydrogenase activity in all transplantable strains was low during the entire duration of culturing. Intensity of the reaction in trypsin-treated SOZ cells was equal to that found in cells removed from the glass by conventional methods (Fig. 3b).

Succinic dehydrogenase activity in the 2nd generation cell suspension of renal tissue was relatively high. Only a few individual cells did not contain a precipitate. It was noted that during the 1st day of culturing the subculture cells showed only an insignificant decrease in enzymatic activity. However, by the second day the activity began to increase and remained at this relatively high level up to the end of culturing. The endogenous activity level of dehydrogenases in the cell suspension was considerable during the first 5 to 6 days, then began to decrease somewhat. The granules of diformazan precipitate in the cells of subculture were small, and attained larger size only on aging.

It was proposed that a reversible decrease in alkaline phosphatase and succinic dehydrogenase activity in the early phases of 1st generation renal cell culture and an absence of such a decrease in the cells of NEr-2 strain could be explained by adaptation of cells to conditions of tissue culture [2, 3]. These observations were confirmed in the present study on a number of different tissue culture strains. The results showed that changes in activity of the enzymes studied were similar in various tissue culture strains. Dynamics of enzyme activity in these cultures corresponded to those of mitotic activity. During the first days of culturing of monkey renal tissue cells and human amnion the mitotic index was 1 to 2‰. Only in the latter stages did it reach 25-35‰ [1]. In the transplantable cell strains it already reached 50 to 70‰ during the first two days of culturing [1].

The fact, first reported by L. G. Stepanova [4], that only a small percent (7 to 8%) of primarily explanted cells was attached to glass while transplantable strains showed 95 to 100% attachment could be explained by various degrees in adaptation. A sharp decline in enzymatic activity and in mitotic index during the early stages of culturing of primarily explanted tissues could be explained by a specific action of trypsin, used in obtaining these cultures. However, absence of any discrepancies in parallel experiments employing trypsin and versene on SOZ cells spoke against it. Numerous reports of studies employing trypsin solution in making passages of transplantable strains likewise did not mention retardation in cellular growth [6, 8, 9].

It was previously reported [5, 10, 11] that transplantable liver cells of Chang's strain, when compared with parenchymatous liver cells, lacked several enzymes. This phenomenon was explained on the basis of cellular dedifferentiation and primitive metabolism in the tissue culture environment. In this connection our findings of sharp rise in alkaline phosphatase and succinic dehydrogenase activities in transplantable amniotic cells as compared to a culture of primarily explained amnion are of particular interest. It is possible that during the process of cellular adaptation to tissue culture environment, concomitantly with disappearance of some enzymatic system activities there occurs a sharp rise in others.

Reported factual material completely confirms the data of Barlington [7], who found a considerable decrease in activity of a number of enzyme systems in cells of primarily explanted renal tissue in comparison with the same cells in suspension. This author, as well as we, explained changes in enzyme activity by an altered environment existing in vitro. Present data concerning endogenous dehydrogenase activity in various tissue cultures confirms previously obtained results [3].

Subcultured renal tissue cells occupy an intermediate position between primarily explanted and transplantable tissue cultures. Endogenous dehydrogenase activity in these cells is relatively high, and in this respect they resemble primarily explanted cultures. On the other hand, these cells did not show a sharp decline in enzyme activity, which suggested similarity to transplantable cultures.

SUMMARY

The activity of succindehydrogenase and of alkaline phosphatase was studied in the transplantable strains of amniotic epithelium, NEr-2 and the heart of Macacca cynomolgus, as well as in the first generation cells of amnion and the second generation cells of the monkey kidney. In difference from the primarily explanted amniotic cells, the activity of the enzymes does not disappear from the cells of transplantable strains and the second generation of the monkey kidney. The endogenous activity of dehydrogenase was relatively high in the primarily explanted amniotic cells and the cells of the second generation of monkey kidney tissue. A possibility of connection between the changes of the enzyme activity and the processes of cellular adaptation to the conditions of culturing is discussed.

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AN IMPROVED APPARATUS FOR MEASUREMENT OF REFLEXES

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In 1951 one of us in conjunction with P. N. Karpenko constructed and subsequently (1954, co-author P. I. Rumyantsev) improved a radioelectronic apparatus for the direct and radiotelemetric measurement of conditioned and unconditioned reflexes [1, 2, 3]. This apparatus (the telechronoreflexometer, i.e., the TKhR-56 radioreflexometer) successfully passed its tests and was recommended by the Technical Committee of the Ministry of Health of the USSR for serial production, which began in 1957. The apparatus was demonstrated at the Exhibition of Achievements of the National Economy of the USSR and at several Soviet exhibitions abroad [5, 6].

In 1958-59 the TKhR-56 apparatus was improved by the authors of this article as a result of further progress in radioelectronics. The new apparatus, with its important additional possibilities, was named the RRM-59 radioreflexometer.

Like the TKhR-56, the RRM-59 apparatus can be used for the investigation of conditioned and unconditioned reflexes in man and animals to both direct and verbal stimuli, for example motor, verbal and respiratory reactions in various combinations, as well as tendon, blinking, defensive, swallowing and certain other reflexes.

The apparatus may be used to determine the latent period and magnitude of a reaction, for example the sound intensity of a vocal reaction. It is possible to make a relative measurement of two different reactions to one stimulus, for example motor and verbal, or motor and respiratory. In conjunction with a radio link the radioreflexometer can be used for radiotelemetric investigations.

In order to measure the latent period of a reaction the apparatus is fitted with a time-measuring device, which is set in motion at the same time as the experimenter gives the stimulus signal, and is switched off at the moment the subject's reaction begins. The possibility of attachment of an oscillograph is foreseen.

The RRM-59 radioreflexometer possesses the following essential advantages over the TKhR-56 apparatus:

1. It is possible to measure not only the latent period but also the duration of the reaction.

2. The new radioreflexomter possesses a much greater accuracy of measurement (to 0.0001-0.0005 second).

3. It ensures the completely inertialess application of signals and makes the working of the time-measuring device independent of the duration of closure of the contacts.

4. The apparatus is completely silent in operation.

5. It can be used to measure the intervals between the most widely differing biological impulses, for example between pulse beats.

6. The RRM-59 apparatus may be used for remote speech control, i.e., for remote control by various electrical effector mechanisms by means of speech (and also by the voice or expiration).

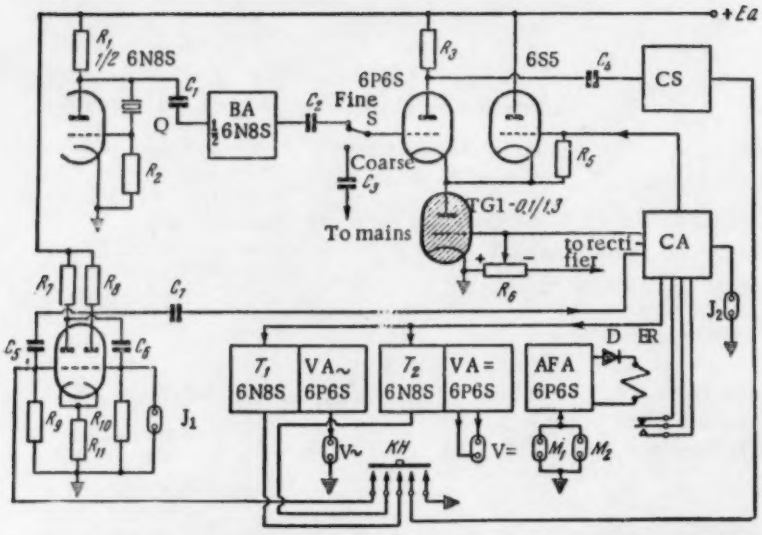

Fig. 1. Simplified scheme of the principle of the radioreflexometer. T_1 and T_2) triggers with two stable states of equilibrium; $VA_{\sim}$) alternating voltage amplifier; $VA_{=}$) constant voltage amplifier; AFA) audiofrequency amplifier; D) detector; ER) electromagnetic relay; CA) commutating apparatus; CS) counting system; BA) buffer amplifier; S) switch; Q) quartz; J_1) and J_2) jacks for coupling contact devices; M_1 and M_2) jacks for coupling microphones (laryngophones); $V_{\sim}$ and $V_{=}$) jacks for output of alternating and constant current.

The principal units of the radioreflexometer are a source of stable oscillations, a counting system, a commutating apparatus and a source of current (Fig. 1).

The source of stable oscillations consists of a master oscillator with quartz frequency stabilization. The counting system used in the apparatus is that of the type MSK-2 millisecond counter, assembled on tubes of types 6N8S, 6N9S and miniature cold thyratrons of the MTKh-90 type. The commutating apparatus consists of a tube relay-amplifier, an auxiliary tube, three triggers, each having two stable states of equilibrium, an audiofrequency amplifier, a detector, an electromagnetic relay and two voltage amplifiers. The tube relay-amplifier consists of an amplifier and a thyratron, included in the cathode circuit of the amplifier.

All units of the apparatus are supplied with current from a source consisting of a power transformer, a type 5Ts4S kenotron and three SG-4S gas stabilizers.

In order to measure the period of visible (the "visible" period of the reaction) feeding or the cessation of feeding of oscillations to the counting system, a change is made in the working conditions of the thyratron, as a result of the inclusion of an area of the grid-mass of the auxiliary tube by the electromagnetic relay during the complete "visible" period of the reaction. During the measurement of the latent period of the reaction the feeding or cessation of feeding of stable oscillations to the counting system is brought about by the action of verbal, respiratory and other reactions, transformed into electrical reactions, on the control grids of the thyratron and auxiliary tube or by closure of a section of the grid-cathode of the trigger with a contact device.

The new reflexometer can be operated in five ways:

1. The time-measuring apparatus (counting system) and stimuli are switched on and off by closure of the contact devices.

2. The counting system and stimuli are switched on by closure of contact devices and switched off by the beginning of the verbal reaction.

3. The counting system is switched on by the beginning of the verbal signal and switched off by closure of the contact devices.

4. The counting system is switched on by the ending of the verbal signal and switched off by the beginning of the verbal reaction. (In this variant the intervals between the pulse beats can be measured).

5. The counting system is switched on by the beginning and off by the ending of the verbal reaction, i.e., its "visible" period is measured. Measurement of the latent and "visible" periods of the same reaction may be carried out jointly in one variant of operation of the apparatus.

In all the variants listed above any electrical effectormechanisms may be switched on or off by the beginning or ending of a spoken word, and in the fifth variant they may be switched on for the period of pronunciation of a word (or a sentence). In the second and fifth variants it is possible to measure, besides a verbal reaction, motor and other reactions.

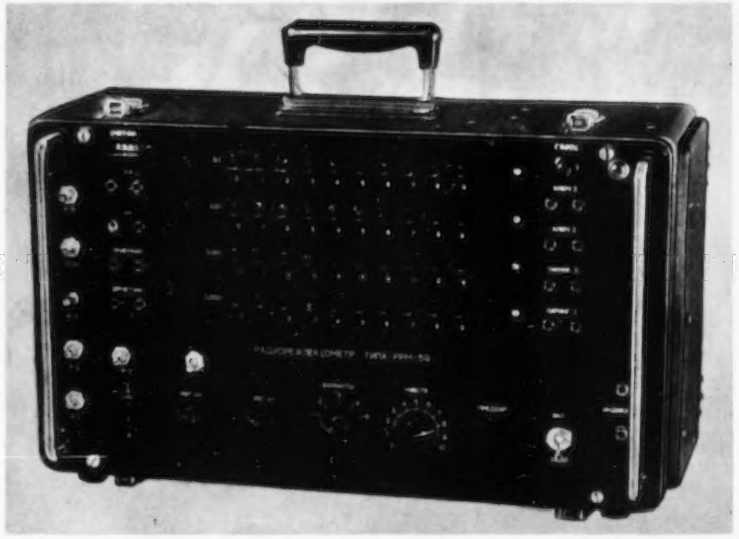

Fig. 2. The RRM-59 radioreflexometer. Front view.

The choice of variants of operation of the apparatus is made by means of a disk-coil changeover switch.

The complete apparatus is mounted in a metal box. On the front panel (Fig. 2), covered with a removable lid, are arranged all the necessary controls: the electromechanical counter, counting decades and jacks for coupling a radio receiver, an oscillograph, an electromagnetic marker, sources of stimuli, contact devices, laryngophones and other pick-up devices.

The apparatus is designed to be supplied from the ac mains at a voltage of 220, 127 or 110 v and a frequency of 50 cps. Its dimensions are 470 x 280 x 220 mm and its weight 10.9 kg. The radioreflexometer is delicate in operation and simple to operate.

SUMMARY

A brief description of a new radioreflexometer is presented. It is an instrument for direct and radiotelemetric measurement of various conditioned and unconditioned reflexes, including motor, speech and respiratory ones.

The device is capable of measuring not only the latent but also "visible" periods of reactions. It may be used for the T. V. speech control by means of the electrical operating mechanisms.

The main parts of the radioreflexometer are: a master generator with a quartz frequency stabilizer, recomputation scheme and commutating device, consisting of several triggers, thyratron and other elements.

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EXCLUSION OF HEARING AND DESTRUCTION OF THE VESTIBULAR APPARATUS IN THE RABBIT

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The conditions of an experiment often demand the exclusion of hearing or of vestibular function in animals. The operative technique of destruction of the cochlear and vestibular apparatus has been well developed experimentally by several workers. Existing methods include a stage of operative approach to the fenestra rotunda or fenestra ovalis, followed by the chemical or mechanical destruction of the vestibular and auditory apparatuses.

However, these methods, although readily available to experimental otolaryngologists, are not so easily carried out in the conditions prevailing in general physiological and pathophysiological laboratories.

The anatomical peculiarities of the position of the cochlea and the vestibular apparatus in rabbits are most unfavorable for a ventral operative approach. For access to the bulla it is necessary to operate at a great depth, the bulla lies in an inconvenient plane, and the approach to it is hindered by the lower jaw. The lateral approach and operative access to the middle ear through the auditory meatus are also complicated and traumatic.

We suggest a method of access to the fenestra ovalis of the vestibule of the rabbit which avoids the necessity of operation. This method may be used when the experimeter requires complete exclusion of hearing or vestibular function.

The oblique tip of an injection needle 0.6-0.9 mm in diameter is sawed off at a right angle. The tip of the needle is then bent to an angle of 90° over the flame of a burner so that the length of the bent portion is 3-5 mm. The external auditory meatus is painted with alcohol. The needle and a syringe are sterilized. The rabbit is not under general anesthesia. The animal is tied down to the bench and its head fixed in the lateral position. The auditory meatus is illuminated and, under direct visual control, the tip of the needle is passed along its internal wall to perforate the tympanic membrane and enter the cavity of the middle ear. The tip of the needle seems to slide off the crest of the lower plate of the upper bony wall of the external auditory meatus. The experimenter at this moment directs the tip of the needle downward with gentle pressure. As a result the needle enters the fenestra ovalis. This gives rise to a sensation of a characteristic crackle, resembling that made by puncturing parchment. After passage of the needle through the fenestra ovalis the second stage of the operation is carried out. This has been well developed by previous workers and is widely employed in practice, and consists of the injection of 96° alcohol and mechanical destruction of the internal ear by means of rotary movements of the tip of the needle. The animal develops spontaneous nystagmus with a rapid component towards the healthy side, the position of the head is changed, and other characteristic disturbances of function associated with unilateral labyrinthectomy appear. The performance of a similar operation on the opposite side abolishes the manifestations of unilateral destruction of the labyrinth and gives rise to the signs of loss of function of both labyrinths (shaking movements of the head and ataxia of the gait) and total loss of hearing.

The entire procedure of bilateral exclusion of hearing and vestibular function takes only 5-10 minutes and is within the competence of any experimenter.

Trial of this method on a large number of animals (over 200) has demonstrated its realiability and convenience. Physiological investigations on rabbits on which this operation has been performed have shown the complete loss of their hearing and vestibular functions. Control morphological examinations on sacrificed animals have shown that complete destruction of the vestibule, the ampullary part of the membranous labyrinth and part of the cochlea takes place. The injection of alcohol supplements the mechanical trauma and leads to the development of connective tissue in the part of the cochlea and vestibular apparatus which remains.

SUMMARY

The authors suggest a method for exclusion of hearing and destruction of vestibular apparatus in rabbits, without any preliminary surgical approach to the fenestra rotunda or ovalis, chemical and mechanical destruction of the cochlea and semicircular canals being carried out with the aid of a special bent needle.

AN ELECTRIC THERMOMETER FOR MEASUREMENT OF THE SKIN TEMPERATURE

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(Presented by Active Member AMN SSSR V. V. Parin)
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Many methods have been suggested for measurement of the skin temperature, based on the use of special mercury thermometers, thermocouples, resistance thermometers and semiconducting resistance thermometers (thermistors) [1, 2]. Defects in these apparatuses and the difficulty of obtaining them have compelled us to seek methods of producing an apparatus which can be made in hospital workshops.

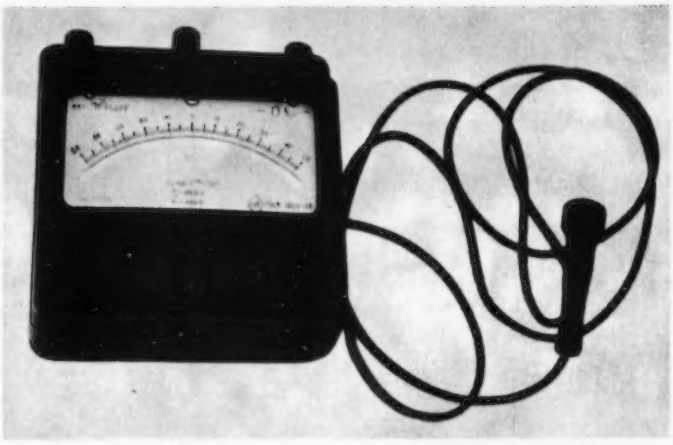

Fig. 1. General view of the apparatus for measurement of the skin temperature.

The greatest difficulty is experienced in the construction of a sensitive pick-up for the thermometer. In this apparatus we therefore used a semiconductor point-contact diode type D1B, of Soviet manufacture.

Investigation of semiconductor diodes showed that the strength of the current flowing through the diode changes with a change in its temperature, and also that these changes are greatest in the region of positive temperatures of 30-40°, i.e., within the range of measurements required in medical practice. Sudden and frequent changes in the temperature of the D1B diode do not lead to appreciable changes in the accuracy of the readings given by the apparatus which we have constructed.

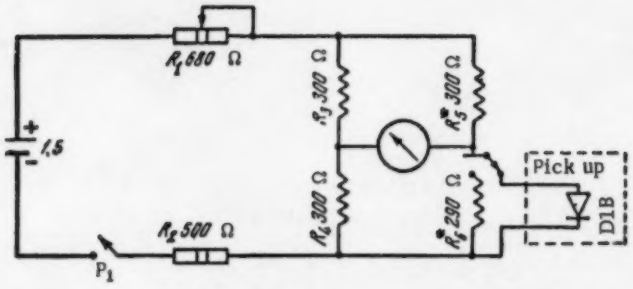

Fig. 2. Scheme of the apparatus for measurement of the skin temperature.

A general view of the apparatus and the scheme of its construction are shown in Figs. 1 and 2.

Our suggested thermometer is assembled according to the principle of a bridge scheme with a type M 117-2 measuring apparatus graduated between 30 and 40°. One arm of the bridge consists of the D1B crystal diode. The thermal inertia of the apparatus with this type of pick-up is 10-15 seconds. The bridge is supplied from a 1.45 v dry cell. The circuit includes a device for ensuring consistent readings of the thermometer irrespective of the voltage of the cell. Our investigations have shown that the accuracy of measurement of the temperature lies within limits of ± 0.1°.

The thermometer which we recommend may be assembled from readily available radio components.

SUMMARY

The author describes an electric skin thermometer furnished with a bridge circuit and a semiconductor diode D1B as a transducer. The precision of the instrument lies within the range of ± 0.1°C.

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A METHOD OF ELECTIVE STAINING OF ACID (SULFATED) MUCOPOLYSACCHARIDES WITH BASIC BROWN*

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The acid mucopolysaccharides are the object of the increased attention of both biochemists and morphologists. Nevertheless the existing methods of detection of acid mucopolysaccharides in histological preparations are insufficiently effective. For instance, metachromatic staining with the thiazine dyes, which plays a considerable part in the study of the acid mucopolysaccharides [13, 3], does not exhibit specificity, for it has recently been shown that metachromasia is given by high polymer phosphates and nucleic acids beside acid mucopolysaccharides [14, 8, 2].

Hale's method, which is so often used for detection of acid mucopolysaccharides, like its modifications, is not specific, for it reveals nucleic acids, lecithin, sphyngomyelin, fibrinogen and other substances in addition to acid mucopolysaccharides [3, 10, 7].

Abroad in recent years alcyan blue has been used widely as a stain for acid mucopolysaccharides [18], although it has not become popular in the Soviet Union because the dye is in extremely short supply. It must be pointed out, however, that the detection of acid mucopolysaccharides by alcyan blue is hardly a specific procedure, for when sections are stained with this dye elective staining of the acid mucopolysaccharides takes place only after exposure for 10-40 seconds, after which other structures not containing acid mucopolysaccharides very quickly begin to take up the stain [3]. This shows beyond doubt that alcyan blue combines not only with acid mucopolysaccharides but also with other substances.

Our method of elective staining of acid mucopolysaccharides is by means of the dye basic brown** (Bismarck Brown, vesuvine). Although the ability of basic brown to stain nuclei, cartilage and mucus has been known for some time [4], this stain has not been widely adopted in histological practice. We have previously described a special technique for the elective staining of mast cells by means of basic brown [6].*** Later we showed that when this method of staining is used, besides the granules of mast cells, other structures take up the stain. The common feature of all the histological elements staining by this method is that they contain acid mucopolysaccharides.

Any form of fixation which preserves acid mucopolysaccharides may be used (absolute alcohol, alcohol-formalin, etc.). Especially good results are given by Shabadash's No. 1 neutral fixing agent (copper nitrate 1.8 g, calcium nitrate 0.9 g, alcohol 100 ml, concentrated formalin 10 ml). The virtues of this fixing agent are explained by results [15] showing that acid mucopolysaccharides are rapidly precipitated by alcohol in the presence of ions of the heavy metals. Em bedding in paraffin wax is carried out by the usual method, so that film preparations can be made.****

* The paper was presented at the Conference on the Histochemistry of the Polysaccharides in Moscow, 1960.

** Its nomenclative number in the Union Reagent Catalog is 1863.

*** Unfortunately the work of Hardy and Wesbrook [9], who used Bismarck Brown to stain most cells, was not known to the authors at the time when they developed the method.

**** The method which is being described was not tested in celloidin and frozen sections. We consider that it should be possible to stain acid mucopolysaccharides in frozen and celloidin sections.

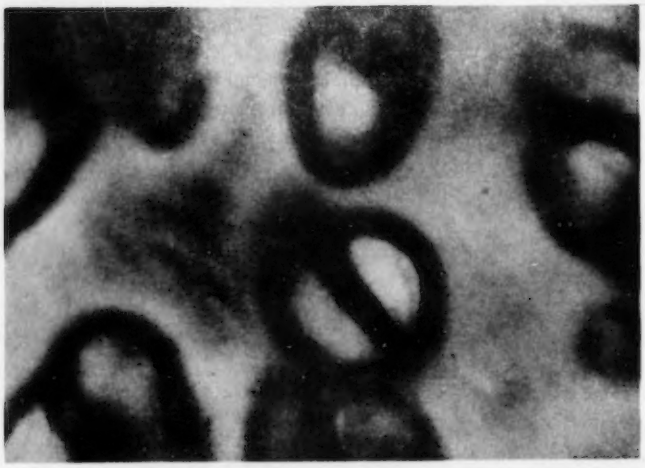

Fig. 1. Hyaline cartilage of the rabbit's trachea. Structures of the ground substance are revealed but the cartilage cell are not stained. Basic brown without counterstaining. Photomicrograph. Magnification: objective 100 x, ocular K-12.

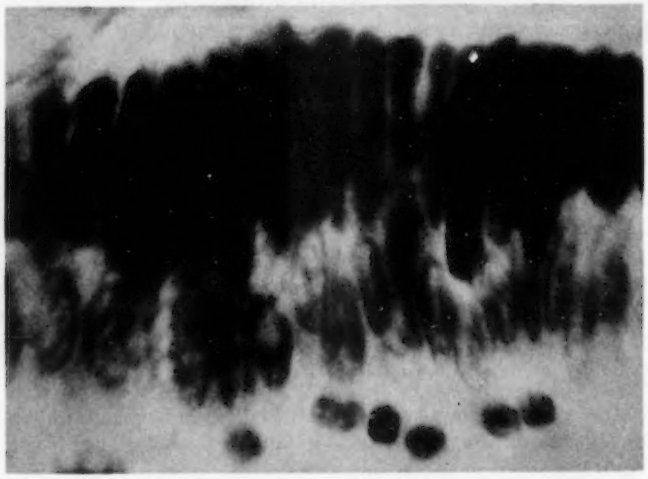

Fig. 2. Mucous membrane of a rabbit's stomach. Mucoid in the apical part of the epithelial cells covering the mucosa is stained with basic brown and the nuclei are counterstained with gallocyanin. Photomicrograph. Magnification: objective 100 x, ocular K-12.

Deparaffinized sections and fixed films are taken through to 70° alcohol and placed in a solution of the dye for 1-2 hours (in most cases 1 hour is sufficient). The dye solution is prepared by the following formula: basic brown 0.5 g, ethyl alcohol 80 ml, 1 N hydrochloric acid 20 ml.* Before it is used for the first time the solution must be filtered. After removal of the preparation from the dye it is taken through three changes of 70° alcohol, for one or two minutes in each, after which it is dehydrated, cleared and mounted in balsam.

* The introduction of a certain amount of hydrochloric acid is important in principle for it ensures the elective staining of the acid mucopolysaccharides by removing the stain from other basophilic substrates.

When this staining method is used an intensive brown coloration is shown by the ground substance of cartilage, the mucoid material secreted by the surface epithelium of the stomach, the mucin in the submandibular and sublingual salivary glands, the mucin in the goblet cells and mucous glands of the trachea, the mucin in the goblet cells of the small and large intestine and the efferent ducts of the pancreas, the cytoplasmic granules of the mast cells and the chromotropic substance of the blood vessels (Figs. 1, 2 and 3). All the structures enumerated above are known to contain different acid mucopolysaccharides containing a sulfate group [1, 4, 3, 15].

Fig. 3. Epithelium of a rabbit's trachea. Mucin in the goblet cells and on the surface of the mucous membrane is stained with basic brown, without counterstaining. Photomicrograph. Magnification: objective 100 ×, ocular K-12.

Other basophilic substances, including nucleic acids, are not stained by this technique. For instance, the nuclei of cells containing deoxyribonucleic acid remain colorless. The tigroid of the neurons and the basal part of the cytoplasm of the secretory cells of the pancreas, which contain ribonucleic acid, also are unstained. The volutin of bacteria (we investigated the diphtheria bacillus and the bacterium of lactic acid fermentation), which gives a marked metachromasia when stained with thiazine dyes and consists, as shown recently [2], of high-polymer metaphosphate, is not stained by our method. No staining is shown by neutral polysaccharides, for example liver and muscle glycogen, or by the mucopolysaccharide secreted by the cells of the neck of the fundal glands of the stomach, which according to data in the literature [15] is not acid in character.

At present it is difficult to give full details of the chemical basis of the staining of acid mucopolysaccharides with basic brown. There is no doubt that a fundamental feature is the combination of the dye with acid groups of the acid mucopolysaccharides. The absence of staining of other basophilic substrates shows that the acid groups of these substrates (the phosphate groups of nucleic acids, the carboxyl groups of proteins) do not combine with basic brown. Comparison of the results of staining with data relating to the chemical composition of the stained substances suggests that the acid group which combines with basic brown is the sulfate group esterifying the mucopolysaccharide. This group is in fact present in all the substrates taking up the stain. Acid mucopolysaccharides not containing a sulfate group are not stained by this method. An example of this is hyaluronic acid, present in the ground substance of connective tissue, which does not stain with basic brown. Control experiments in which sections were treated with hyaluronidase (the preparation "lidase" in a concentration of 1 mg/ml, 3 hours at 37°) showed that hyaluronic acid does not participate in staining with basic brown.

In order to verify our hypothesis that staining is related to the presence of a sulfate group in the mucopolysaccharide molecule in an ester bond, experiments were carried out involving methylation and subsequent saponification of the sections. The method of treatment was as follows: 1) Methylation (deparaffinized sections were kept for 2 hours at 60° in methanol containing 0.1 g. eq. hydrochloric acid); 2) Saponification (after methylation, the sections were kept for 20 minutes at room temperature in a 1% solution of caustic potash in 80° ethanol).

During treatment of the sections with methanol containing 0.1 g. eq. hydrochloric acid, a methanolytic "desulfatation" of the acid mucopolysaccharides takes place, i.e., the irreversible splitting of the sulfate group from the mucopolysaccharide to form a polysaccharide free from sulfate. If the above-mentioned experimental

conditions are observed, nucleic acids are not affected by methylation and their staining properties are unchanged. During methylation the carboxyl groups of proteins are esterified, which leads to the disappearance of their inherent basophilia. Saponification causes decomposition of the esters and the reappearance of basophilia, due to the carboxyl groups of the proteins [11, 12, 16, 17].

The experiments show that treatment of sections with methanol containing 0.1 g. eq. hydrochloric acid prevents the staining of all structures staining with basic brown. Subsequent saponification does not lead to the reappearance of staining. These facts give grounds for the assertion that the staining of acid mucopolysaccharides with basic brown is due to the presence of an ester-bound sulfate group in the composition of the mucopolysaccharide.

Our suggested method of staining of acid mucopolysaccharides is characterized by its essential specificity and it gives standard, reproducible results. The method is simple in use and does not require reagents in short supply. By combining staining with basic brown with treatment by other histochemical methods (the Feulgen reaction, Shabadash's method, staining with chrome alum-gallocyanin, etc.) it is possible to obtain multicolored preparations in which neutral polysaccharides, nucleic acids and other substances are revealed in addition to acid mucopolysaccharides.

SUMMARY

The author suggests a method of selective staining of acid (sulphated) mucopolysaccharides in histological preparations. Sections or film preparations are passed through alcohols, gradually increasing in grade, finally reaching the 70° grade. Then they are put into a solution of basic brown and left there for 1-3 hours (stain – 0.5 gm, alcohol – 80 ml, 1 N hydrochloric acid solution – 80 ml). This procedure is followed by passing the section through 3 containers with 70° alcohol (1-2 minutes in each), dehydration, clearing and embedding in balsam. Experiments with methylation and subsequent saponification of the sections demonstrate the dependence of the staining upon the presence of mucopolysaccharides of the ether-bound sulphate group in the molecule.

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* Original Russian pagination. See C. B. translation.

DETECTION OF INTERMEDIATE PRODUCTS OF OXIDATION OF LIPIDS IN TISSUE SECTIONS

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It is nowadays possible to detect certain intermediate products of lipid metabolism in tissue sections histochemically. This applies, in particular, to products of oxidation of lipids such as peroxides and aldehydes. The reactions of Schiff and of Glavind [5] are well suited to this purpose.

Schiff's reaction is widely used in histochemistry for the detection of substances capable of forming aldehyde groups as a result of certain forms of treatment. This group includes, in particular, substances such as deoxyribonucleic acid, acetalphosphatides and polysaccharides.

The property of restoring the color of the dye fuchsin-sulfuric acid (leukofuchsin) is possessed by bromine and by certain ketones [2, 3, 7, 12] in addition to aldehydes. The possibility of the participation of these substances in the reaction is, however, doubtful. The solution of this problem is important to the histochemistry of the ketosteroids. Oster [9, 11] is inclined to regard Schiff's reaction with ketones as a "pseudoreaction," because the product formed is rose pink in color and not the typical purple. At the same time Oster and Mulinos [10] have shown that the product of the reaction between tissue aldehydes and fuchsin-sulfuric acid can be decolorized with alkalis and the initial color restored with acids. This type of result is unattainable with the pink-staining product of combination of fuchsin-sulfuric acid with ketone. They suggest the procedure of successive alkalization and acidification as a test for the more precise identification of a substance detected by means of Schiff's reagent. Lison [8] pointed out some time ago that a reaction with fuchsin-sulfuric acid may also be given by unsaturated fatty acids and that this reaction involves ethylene groups. Subsequent investigations, however, have shown these claims to be unfounded. It has been shown that oleic and other unsaturated fatty acids are able to react with fuchsin-sulfuric acid only after preliminary oxidation and the formation of aldehyde groups [2, 11].

There are claims in the literature that the oxidation of unsaturated fatty acids, especially in the presence of light and heat, takes place relatively rapidly [6]. This arouses misgivings that the formation of aldehydes from unsaturated lipids may take place in the process of histological treatment of the material. In order to test this hypothesis we carried out the following experiments.

Pure oleic acid was injected subcutaneously into rats; three hours later the animals were sacrificed and the skin at the site of injection was excised, together with the subcutaneous cellular tissue and muscle, and immersed in 10% formalin. After fixation for 24 hours the material was rinsed in tap water and embedded in gelatin by the usual method. Sections were cut with the freezing microtome and Schiff's reaction carried out with them, after which they were stained with Nile blue sulfate.

In all cases the results of the Schiff reaction were negative. After staining with Nile blue, cavities containing oleic acid, staining a dense blue, were clearly seen in the subcutaneous fatty tissue. The experiments

thus showed that oleic acid does not give a reaction with fuchsin and sulfuric acid, and that in the process of the usual histological treatment and embedding in gelatin decomposition of oleic acid and formation of aldehydes do not take place.

Experiments in which Schiff's reaction was performed on sections of normal skin and subcutaneous cellular tissue also gave negative results. Consequently, the lipids of normal skin do not contain aldehydes, and in the process of histological treatment of the material aldehyde formation does not occur. Prolonged storage of the material in the air or in formalin leads to the formation of both carboxyl (giving a blue stain with Nile blue sulfate) and aldehyde groups (positive reaction with Schiff's reagent). The histochemical study of intermediate products of oxidation of lipids thus requires that the material used should be fresh or fixed in formalin for not longer than 24-48 hours.

Schiff's reaction has been used as an indicator of the decomposition of lipids in experiments in vitro by Fellenberg [4], Inikhov [1] and others.

Glavind's [5] method has been suggested comparatively recently, and it consists of the oxidation of leuko-2-6-dichlorophenolindophenol with tissue peroxides with the formation of indophenol, which is readily soluble in lipids and is shown by its red color. To accelerate the reaction a solution of hemin in a mixture of pyridine and glacial acetic acid is used as a catalyst. Leuko-2-6-dichlorophenolindophenol is prepared from sodium 2-6-dichlorophenolindophenolate by reduction with ascorbic acid.

This method has been tested by us in vitro with hydrogen peroxide, with oleic acid, with a solution of oleic acid in ethyl alcohol, with formaldehyde, and also in tissue sections from rats receiving preliminary subcutaneous injections of oleic acid before sacrifice. The problem of whether unsaturated fatty acids and aldehydes may give a positive reaction was of great interest, for it indicated the specificity of the method. The results obtained are given in the table.

Comparison of the Results of the Reaction of Fatty Acids, Aldehydes and Peroxides with Glavind's Reagent and with Sodium 2-6-Dichlorophenolindophenolate

Material	Results of reaction	
	with Glavind's reagent	with sodium 2-6-dichlorophenolindophenolate
Hydrogen peroxide	Red	Red
Oleic acid	Not stained	Red
Solution of oleic acid in ethyl alcohol	Not stained	Red
Formalin	Not stained	Blue
Oleic acid injected subcutaneously	Not stained	Red

Of all the substances which we investigated, only hydrogen peroxide thus gave a stain. Neither oleic acid, as representative of the unsaturated fatty acids, nor formalin, as representative of the aldehydes, gave staining with Glavind's reagent. The findings that Glavind's method reveals peroxides but not aldehydes and acids are also confirmed by model experiments in which we compared the results of staining the lipids of the skin and subcutaneous cellular tissue after preliminary oxidation by means of three methods: Nile blue sulfate, fuchsin-sulfuric acid and Glavind's method.

During microscopic investigation of this material it may be observed that the distribution of the areas giving a positive reaction with each of these methods does not always coincide.

We know that 2-6-dichlorophenolindophenol is an indicator giving a red color in an acid medium and a blue color in an alkaline. Analysis of our findings (see table), however, shows that the acid-alkaline color changes

characteristic of 2-6-dichlorophenolindophenol are by no means so of its leuko-form (Galvind's reagent). Consequently, the color changes observed cannot be attributed to changes in the pH of the medium.

When Galvind's reaction is carried out with normal human skin, the fat cells of the dermis and the subcutaneous cellular tissue acquire a weak pale pink color. This weak staining is evidently the result of the presence of a small quantity of peroxides, formed in the lipids during the time elapsing since the moment of death. The fat cells of the skin and of the subcutaneous cellular tissue of the rat, investigated directly after decapitation of the animal, do not show this coloration.

Our observations showed that it is unnecessary to obtain the pure leuko compound in a dry form. In the reaction it is quite suitable to use solutions of this compound obtained ex tempore by the reduction of an alcoholic (50° alcohol) solution of sodium 2-6-dichlorophenolindophenolate by means of addition of ascorbic acid (also in the form of a solution in 50° alcohol). To preserve the relative proportions of the individual ingredients in the reagent mixture as recommended by the authors, it is better to use a 0.3% solution of sodium 2-6-dichlorophenolindophenolate. The concentration of ascorbic acid is not of fundamental importance, but it must be high enough to produce minimal dilution of the mixture as a result of its addition.

According to the results of the authors of the method [5], excess of ascorbic acid may retard the reaction, so that addition should be made carefully, a drop at a time, precisely to the moment when the blue-green color changes to brown. To 4 ml of the resulting mixture is added 0.35 ml of a previously prepared solution of hemin in pyridine and glacial acetic acid (hemin 40 mg, pyridine 10 ml, glacial acetic acid 20 ml; this solution is stable and may be kept for a long time).

Sections are laid out on slides and dried with filter paper to remove excess of water, in order to prevent loss of hemin. The mixture of reagents is poured onto the sections for 3-5 minutes. The sections are then thoroughly washed in distilled water and examined immediately (they may be mounted in glycerol), for the reaction is unstable and the color disappears in about one hour. Material containing peroxides gives a red color.

SUMMARY

Schiff's reaction (for aldehydes) and Glavind's reaction (for peroxides) may be used for histochemical detection of intermediate products of fat oxidation. The question of the specificity of these methods is discussed and the technique of conducting these reactions on tissue sections is presented on the basis of literature data and the author's investigations.

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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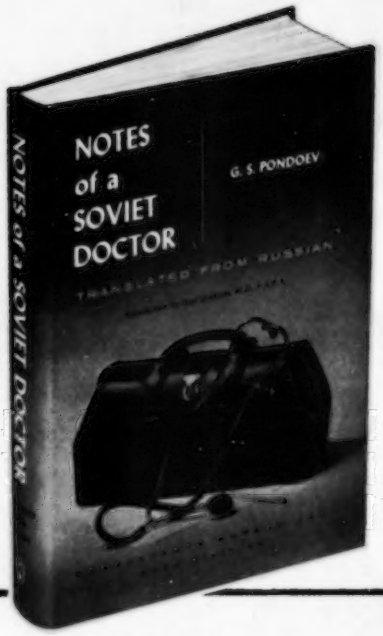

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